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# Dried poultry waste and non-protein nitrogen for poultry

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# Dried poultry waste and non-protein nitrogen for poultry

by

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# INTRODUCTION

The high price of feedstuffs, especially of nitrogen sources, has been one of the most serious problems facing animal and poultry producers since man knew how to trade. One of the main purposes of research in the nutrition field is to reduce the cost of animal production directly or indirectly. It is interesting to notice that as the world progresses, the farming area becomes smaller. A smaller farming area does not necessarily affect the quantity of animal production but it limits the area of animal waste disposal. The above reasons together with the increasing public awareness of environmental pollution urge investigators to pay considerable attention to the re-utilization of livestock and poultry wastes as feedstuffs for farm animals and poultry. Since animal and poultry wastes contain a high percentage of non-protein nitrogen (NPN), the utilization of NPN is also seriously investigated.

It is well known that ruminants can utilize NPN and dried poultry waste (DPW) efficiently, but NPN and DPW utilization by poultry species is still doubtful, since poultry lack the capacity for microbial fermentation and for microbial synthesis of proteins and amino acids at sites in the alimentary tract which permit their subsequent digestion and efficient absorption. However, there are reports in the literature which indicate that NPN and DPW could be utilized by poultry to some extent.

Utilization of DPW as a feedstuff could be very beneficial in countries where sun-drying of poultry waste is possible throughout the year,

since parametric linear programming has shown that DPW had a value of not more than $26.00 per ton and the costs, including capital investment for equipment plus fuel and labor to dry poultry waste, was estimated at $20.00 to $30.00 per ton of DPW.

Six experiments were carried out to investigate the possibilities of utilizing DPW and NPN as poultry feedstuffs. Attempts to improve the DPW and NPN utilization by poultry were also conducted. Since an ultimate goal in poultry production is a closed circuit system in which the only by-product of the system is meat or eggs, the possibility of DPW re-cycling was also investigated.

# REVIEW OF LITERATURE

## DPW for Young Chicks

The major nitrogenous compounds in urine from chicks have been determined by numerous investigators, (Paton, 1910; Sharpe, 1912; Mayrs, 1924; Davis, 1927; Coulson and Hughes, 1930; Edwards and Wilson, 1954) but the results are highly variable. In 1960, O'Dell et al. determined the nitrogenous composition of ureteral urine from male White Leghorn chicks 5 to 6 weeks of age. The birds received a practical corn-soy meal diet and others received purified diets containing casein, casein and gelatin, casein and supplemental arginine, and liver protein as sources of protein. They reported that uric acid made up about 81% of the total nitrogen of urine and ammonia about 10%. There was some variation of these components with diet but the combined percentage was quite constant. The proportion of urea increased when arginine was added to the diet, but the creatine-creatinine nitrogen and the distribution of amino acids were not affected by diet. Amino acid nitrogen made up about 2% of the total urinary nitrogen. The chief amino acids found in chicken urine, in order of decreasing concentration, were glycine, proline, glutamic acid, lysine, ornithine and arginine. Although hydroxyproline and glucosamine had not been reported in mammalian urine, they were found in all samples of chick urine.

The above reports show that urine, which is part of dried poultry waste (DPW), could contribute nutritional value to DPW for chicks.

Yushok and Bear (1943) observed that old litter from laying hens contained 2.5% nitrogen on an oven-dried basis. This was equivalent to 15.63% crude protein. Fresh hen manure contained the nitrogen equivalent of 25.87% crude protein. White et al. (1944) reported that dried, fresh hen manure contained 35.12% crude protein. Parker et al. (1959) obtained samples of poultry litter from 31 hen houses and 82 broiler houses and found their average crude protein content was 12.5 and 15.28% respectively.

Several groups of investigators have reported the "unidentified growth factor" activity of various types of manure when used in poultry rations. Whitson et al. (1945) reported unidentified growth factor activity in cow manure. Rubin et al. (1946) reported that the chick growth factor present in cow manure was shown to be present in the droppings of hens. Since the growth substance was not in the hens' diet, they suggested that growth factor synthesis must be present. They also reported that urine-free feces of hens stimulated considerably more rapid growth in chicks than did the normally voided mixture of feces and urine. The droppings from growing chickens (3-6 weeks of age) did not contain measurable amounts of the growth factor. Elam et al. (1954) added an autoclaved water suspension of poultry litter to a corn-soybean meal basal diet supplemented with recommended levels of the necessary vitamins and minerals. Growth was increased by the addition of the litter preparation, fish solubles, or an antibiotic combination. Fuller (1956) reported that hydrolyzed poultry litter was as effective as fish meal in supplementing a commercial-type broiler ration under practical conditions. Four chick feeding trials were conducted by Wehunt et al. (1960) to study

the nutritional value of hydrolyzed broiler litter and autoclaved hen and broiler manures as sources of protein and unidentified growth factors for chicks. They found that growth rate of chicks was improved when 1.5 or 3.0% autoclaved manure was added to diets sub-optimal in protein. Feed efficiency was not improved correspondingly and in some instances was actually depressed slightly when the litter or manure was used at 3% in the diet. When used to supplement diets which were sub-optimal in protein, the crude protein of the manure was utilized somewhat less efficiently than that of soybean oil meal or a mixture of casein and gelatin, but on the basis of true protein, the g. protein/g. gain was approximately equal for all supplements. They also showed that about one-half of the crude protein of the hen manure, and one-third of that of the broiler manure used in the protein study, was true protein. They concluded that autoclaved manure appeared to be nearly equal to a combination of fish solubles and dried distillers' solubles, and superior to either alone, in supplementing corn-soybean oil meal type rations containing no other recognized source of unidentified growth factors.

During the last few years, DPW as a feedstuff has been the subject of research of many groups of investigators. Flegal and Zindel (1970c) conducted two experiments to determine the nutritional value of DPW for growing chicks from 1 to 28 days of age. They fed isonitrogenous diets containing 5, 10, 15 and 20% DPW from pullets fed a laying ration. True protein nitrogen of the DPW was fairly constant (1.64-1.79%) within various samples, whereas, the non-protein nitrogen differed rather widely (1.41-3.59%). Their results showed that chicks' body weight gain de-

creased as the levels of DPW in the diet increased, but 5% DPW in the diet did not reduce body weight. Feed efficiency of chicks was inversely related to the levels of DPW in the diet. Weight gain and feed efficiency of chicks fed the 20% DPW diet, however, improved when 4.5% stabilized fat was added to the diet, replacing corn. They suggested that the poor weight gain and feed efficiency of birds fed DPW was due to the low energy content of the DPW. Unfortunately, they failed to mention the energy of either DPW or experimental diets and also they did not mention the protein content of the diets.

A similar result was reported by McNab et al. (1972) who found that chicks given a diet with up to 20% autoclaved dried poultry manure (ADPM) plus maize oil were as heavy as controls at the age of three weeks. At 2 weeks of age only the birds given the diet containing the 20% ADPM performed significantly better than those on the low-protein diet. They suggested that ADPM could be employed in broiler rations to replace some of the protein.

Lee and Blair (1972) described experiments performed with broiler chicks given diets containing crystalline amino acids, (including proline), supplemented with various levels of dried autoclaved poultry manure (DAPM) and uric acid. Better weight gains were obtained from birds fed diets supplemented with DAPM, but uric acid in the diet slightly depressed growth. This result was similar to that of Bare et al. (1964), who found that 1% or more uric acid in the diet of chicks caused growth depression. Lee and Blair (1973) reported that chicks fed a crystalline amino acid diet plus 20.1% DPM had similar weight gain as did chicks fed the diet

plus 12% glutamic acid and gained more than those fed the diet plus essential amino acids. Under field trial conditions, with chicks fed commercial-type diets, 5.0% DPM improved feed efficiency but body weights were unaffected by DPM at 4 weeks of age. At 6 weeks of age, chicks fed 5 and 10% DPM were significantly heavier than the controls, but this increase was not significant at 8 weeks. Food consumption was not affected, but feed efficiency improved with increasing inclusion of DPM.

Burgman et al. (1964) reported that chemical analysis of laying house poultry litter was: moisture, 19.5%; protein, 14.38%; fat, 0.78%; fiber, 16.22%; ash, 22.64%; N.F.E., 26.41%; calcium, 6.07%; phosphorus, 1.77%; ammonium nitrogen, 0.39%; equivalent crude protein from non-protein nitrogen, 2.41%; and gross energy, 360 kcal per kilogram. They also indicated that poultry litter was high in other minerals, low in energy and vitamin A and D.

Metabolizable energy values of DPW obtained by Nesheim (1972) were low and variable. He observed that the DPW from hens fed a diet containing 20% wheat bran had a metabolizable energy value of 90.7 kcal per pound as compared to 217 kcal per pound for dried manure from hens fed a corn-soy diet. On the other hand, manure from another group of hens fed a standard laying diet, but when the manure was air-dried under the cages several weeks before oven drying, had a value of 340.2 kcal per pound. Polin et al. (1971) reported that the metabolizable energy value of DPW was 586 kcal per pound and it increased as the levels of energy in the diet fed to the birds increased. This report was similar to that of Pryor and Connor (1964) who reported that dried feces of 22-day-old

chicks fed a diet containing 80% crushed grain sorghum mixed with 20% feces contained 503 kcal per pound. An air-dried sample of poultry excreta contained 745 kcal per pound in a study reported by Yoshida and Hoshii (1968).

Proximate analysis of dried poultry waste sample done by Polin et al. (1971) showed 24.7% crude protein and 1.86% NPN. They also observed that only 34% of the total nitrogen in DPW was used as a protein source by chickens. A compilation from a number of reports assembled by Blair and Knight (1973) indicated that crude protein contents of DPW varied from 15.2 to 36.8%. The mean value listed for the protein content of poultry battery manure was 28.7% and of poultry house litter was 25.3%. The true protein content was 10.5% for battery manure and 16.4% for the poultry house litter. From 47 to 64% of the nitrogen occurred in a non-protein form and some 30 to 60% of this fraction was uric acid, with small quantities of urea, creatine and ammonia salts. Sheppard et al. (1971) found that there was an inverse relationship between the heat of drying and total crude protein in dried manure. The correlation of drying temperature to the resulting protein approached significance. The coefficient was -0.284; -0.288 would indicate significance at the $P = 0.05$ level. Bose and Ghosh (1945) and Baker (1946) showed that dried poultry excreta contained from 4 to 10% uric acid. Bare et al. (1964) and Blair (1972) indicated that uric acid in DPW was not utilized by poultry. A table presented by Blair and Knight (1973) showed that DPW was high in fiber (13.84% for battery manure and 18.65% for house litter) and ash (26.5 and 14.1%, respectively), and was low in fat (2.0%)

and carbohydrates (6.7% for dried battery manure). However, DPW was a good source of phosphorus and calcium (2.2% P and 7.8% Ca for dried battery manure). Polin et al. (1971) reported that when 9.7% DPW was substituted for corn in the diet of hens, total calcium utilization was 28% as compared with a calcium utilization of 41% for hens fed the control diets. Phosphorus utilization was slightly depressed when 9.7% DPW was included in the diet.

Flegal and Zindel (1970b) and Nesheim (1972) reported a similar amino acid pattern in DPW collected from laying hens. They indicated that DPW appeared to be a relatively poor source of methionine plus cystine.

## DPW for Laying Hens

Many groups have recently investigated the use of DPW as a feedstuff for laying hens. Flegal and Zindel (1969) fed laying hens isonitrogenous diets containing 0, 10, 20 and 40% DPW plus 4.5% added animal fat. They found that hens fed a diet containing 10% DPW laid the most eggs and hens fed the diet containing 40% DPW plus 4.5% animal fat produced the fewest eggs. However, there were no statistical differences in egg production, shell thickness and egg weight. Only Haugh unit scores were found significantly different. A year later, Flegal and Zindel (1970a) conducted a similar experiment and reported that feed efficiency was inversely proportional to the amount of DPW in a ration. An addition of animal fat to the ration resulted in a slight improvement in feed efficiency. Flegal and Zindel (1970b) showed that egg production

on a hen-housed basis decreased as the levels of DPW in the diets increased from 10 to 30%. Levels of DPW at 10 or 20% in the diets did not affect feed per dozen eggs but a 30% level of DPW significantly increased feed per dozen eggs.

York et al. (1970) reported that including 10, 20 or 30% DPW in the diet of hens had no significant deleterious effect on the quality of shell eggs as measured by Haugh units, storage weight loss, color, odor and/or microbial content. Hodgetts (1971) added a little over 10% DPW to a laying hen ration. He observed no differences in egg production or egg size in his feeding trial. He reported that hens fed the diet containing DPW consumed slightly less feed and were 90.7 g. heavier after twelve months of lay. In contrast, Nesheim (1972) reported that hens fed a diet containing 22.5% DPW were significantly lighter than those fed control diet.

Blair and Lee (1973) supplemented 9.7% dried autoclaved poultry manure to a basal diet for laying hens containing 11.5% protein with and without 1.54% essential amino acids supplemented. They reported that egg production, food intake, food conversion efficiency, gross efficiency of nitrogen conversion and the ability of hens to maintain body weight were improved by supplementation with essential amino acids. Supplementation with dried, autoclaved, poultry manure significantly increased food intake, total egg mass and mean egg weight. The gross composition of eggs was not influenced significantly by dietary treatment but albumen quality was significantly lower on the basal diet and higher on the supplemented diets. Nesheim (1972), Young (1972) and Young and Nesheim

(1972) concluded that DPW was an acceptable ingredient for the formulation of laying hen diets when the nutrient content of the ration was balanced. Hens fed diets containing DPW increased feed intake to compensate for the low M.E. content of the poultry waste.

Flegal et al. (1970) fed DPW at dietary levels of 10, 20 and 30% to Single Comb White Leghorn hens for four months before eggs were collected for taste panel evaluation. A cage-type laying diet was used as a control. Eggs from each treatment group were hard-cooked and prepared for a Consumer Preference Panel. They found that dietary levels of DPW fed had no significant effect on the taste of eggs. Panel members were unable to detect any consistent taste difference between eggs from hens fed control diets and those from hens fed DPW supplemented diets.

Flegal and Dorn (1971) fed diets containing 0, 12.5 and 25.0% DPW to laying pullets for about seven months. The DPW were continuously re-cycled for 14 cycles. They found that the proximate analysis of the DPW from each of the experimental rations was similar. There was a trend towards a slight accumulation of calcium and phosphorus. Egg production for the birds fed the control diet and the diet containing 12.5% DPW was similar. There was no significant difference between feed consumption of birds fed the control diet and of birds fed the 12.5% DPW diet, but those birds fed the diet containing 25% DPW ate 11.3 g. more feed per bird per day than those fed the control diet. Varghese and Flegal (1972) conducted a similar experiment and reported that after 23 re-cycles the levels of arsenic acid, mercury, copper and zinc were not appreciably altered in the tissues, feces or eggs by re-cycling DPW

in the diet of laying hens.

Ousterhout and Presser (1971) conducted an experiment to measure the performance and feces production from hens in which total wet and fresh feces production each day was re-cycled back through their feed. They found that egg production was normal for eight days and then decreased rapidly. Feces production increased to 2.5 times that of the control group before feed consumption and feces production plateaued. Re-cycling manure the first time resulted in a 25% utilization of the total dry matter nutrients. The second re-cycling of all the fecal material resulted in a lower utilization. However, the analysis of feces indicated a constancy of dry matter, fiber, ash, calcium, phosphorus, potassium and sodium content. They also suggested that re-cycling manure reduced the disposal problem by no more than 25% with no noticeable further reduction with repeated re-cycling. Re-cycling of DPW as a method of waste disposal was evaluated by Young (1972). He stated that if DPW was re-cycled at 12% or 22% of the diet, the amount of manure which must be handled by other waste management systems would amount to 75-80% of the manure produced by hens fed a standard laying ration. Parametric linear programming showed that DPW had a value of not more than $26.00 per ton and was used primarily as a source of phosphorus in low energy diets. The costs including capital investment for equipment plus fuel and labor to dry manure was estmated at $20 to $30 per ton of DPW.

Couch (1973) indicated that fecal material collected from birds maintained in cages could be fed to laying hens at a level up to 25%

of the diet without detrimental effects. The laying hens could utilize calcium, phosphorus and amino acids in the true protein of DPW. DPW was a low-energy, low-protein potential ingredient. The feeding value was probably in the range of 30-35% of that of corn. Couch concluded that DPW would affect feed conversion adversely on a linear basis as the level in the diet was increased.

## The Effects of Drug Treatments on DPW Utilization

According to Coates et al. (1952), an infectious agent must be present in order for antibiotics to stimulate growth in chicks, since chicks reared in a new, clean environment did not respond to dietary antibiotics. This concept was supported by the work of Anderson et al. (1956) who showed that feeding chlortetracycline overcame the depression in growth rate of chicks fed enterococci. Mameesh et al. (1959) obtained a consistent growth response in chicks fed terramycin when the diet was contaminated with hen feces; a response under these conditions was apparent in only one of six trials with penicillin. Warden and Schaible (1961) fed antibiotics to chicks up to four weeks of age. The ration was contaminated with fresh, dried or autoclaved fecal preparations from caged layers fed a diet containing no antibiotic. They found that a significant growth depression occurred in chicks which had access to fresh hen feces. When the fecal material was dried at 100°F. or dried and autoclaved at 15 pounds pressure for 30 minutes, no growth depression occurred. The addition of zinc bacitracin or aureomycin to a basal ration, not contaminated with fresh feces, permitted the chicks to grow

more nearly at their optimum potential. Growth was significantly improved when either terramycin or aureomycin was fed. Yates and Schaible (1961) found that the addition of antibiotics improved rate of growth and feed utilization when feed was uncontaminated with feces. Fresh hen feces added to the feed depressed growth, except in some lots which received no antibiotics. Virginiamycin gave increased response in growth rate of chicks in batteries as levels were increased (from 4 to 9 to 100 g. per ton), with the best feed utilization obtained with the 9 g. level. The high level of terramycin or zinc bacitracin was not as beneficial as the low levels. Kolar and Seymour (1971) reported that broilers that received spectinomycin, regardless of level or duration of medication, were significantly heavier than the controls. Feed per unit of gain was significantly improved by spectinomycin.

The relationship between the microbiological flora of young chicks and the antibiotic growth effect was explained by Huhtanen and Pensack (1964). They showed that in the unfed day-old chick, a significant bacterial population was found only in the ceca and low ilium. These organisms were mainly Streptococcus faecalis and were replaced in the ceca by other types after feeding was initiated. On the third day, S. faecalis became established in the upper intestine and, at 6 days, reached maximum numbers. Since a malabsorption syndrome occurred in chicks at this age, the relationship of S. faecalis to this phenomenon was examined under gnotobiotic conditions. This organism significantly depressed growth under conditions of monocontamination. An antibiotic, effective against the bacteria, reversed this growth depression. After the sixth

day, S. faecalis diminished in numbers until it was insignificant at 28 days. Coliform bacteria appeared very early in the ceca and remained predominant until obligate anaerobes replaced them at 14 days of age. Lactobacilli were found in the duodenum at early ages and were predominant from 14 days to the end of experiment (28 days).

An investigation was designed by Foster (1972) to evaluate and compare five feed additives: virginiamycin, zinc bacitracin, a nitrofuran derivative, and two arsenical compounds. They found that only arsenicals produced results economically superior to the control diet. The total weight and the weight per unit area of the wall of the small intestine was found to be greater in birds on the control diet than under any of the treatment diets. Stutz and Metrokotsas (1972) reported that fosfomycin at 0.00275 and 0.0055% levels in diets gave statistically increased weight gain and feed efficiency, similarly to the effects of bacitracin in contaminated battery tests. Growth rate and feed efficiency on recycled diets were improved with both antibiotics. Both fosfomycin and bacitracin produced a statistically significant reduction in gut thickening as measured by small intestinal weights.

Suresh et al. (1972) reported tha 3-nitro-4-hydroxyphenylarsonic acid supplemented in the diet at 50 mg. per kg. significantly improved body weight of chicks, but amprolium plus ethopabate at 125 mg. per kg. of diet did not improve body weight. Both amprolium and 3-nitro-4-hydroxyphenylarsonic acid significantly improved feed efficiency.

Antibiotics fed to hens at low levels, under 10 g. per ton, caused no effect on egg production or on any other factors studied in experi-

ments conducted by Berg et al. (1952), Petersen and Lampman (1952), Brown et al. (1953), Carpenter et al. (1954) and Sherwood and Milby (1954). However, when higher levels of antibiotics, 30 to 300 g. per ton, were fed to laying hens, egg production was usually increased. This was reported by Elam et al. (1953), Balloun (1954), Bearse and Berg (1955), Branion et al. (1956), Price et al. (1956) and Ryan et al. (1961). Wilkinson (1961) also reported that zinc bacitracin and oxytetracycline at 100 g. per ton of diet increased production and feed efficiency of laying hens significantly, but had no effect on shell thickness. In contrast, Charles et al. (1971) indicated that terramycin at 0.55 g. per kg. of diet significantly improved shell strength as compared with eggs from hens fed diets with aureomycin or neo-terramycin at the same levels.

Unfortunately, reports on the effect of antibiotics on laying performance when DPW was added to the diet could not be found in the literature.

## Non-protein Nitrogen for Young Chicks

Utilization of non-protein nitrogen (NPN) by chicks is still questionable. There are evidences that non-protein nitrogen compounds can be utilized to a limited extent by young chicks. On the other hand, many research reports show that most NPN compounds are not utilized by chicks.

In 1940, Ackerson et al. conducted an experiment adding urea to supply 13% of dietary nitrogen. They found that urea did not produce any beneficial effect in growing chicks. This was confirmed by the work

of Heller and Penquite (1941). By displacing the protein supplement on an iso-nitrogenous basis with urea at three levels: one third (0.91% urea), two-thirds (1.64% urea) and all (2.24% urea), Bice and Dean (1942) found that chicks fed the test rations showed a severe protein starvation syndrome characterized by retarded growth.

Sullivan and Bird (1957) were the first to suggest that urea or diammonium citrate, when added to low-protein practical type diets, improved growth of chicks. They added urea or DAC (diammonium citrate) to a methionine-supplemented diet and found that chicks fed diets with urea or DAC added had better weight gain than those fed the methionine-supplemented basal diet. They also found that by adding urea to a diet containing sodium glycolate, chick growth was increased over sodium glycolate alone. In contrast, Machlin and Gordon (1957) reported that urea was inhibitory to growth and feed efficiency, and DAC supplementation resulted in a growth response in 2 out of 6 trials.

Subsequent studies in which a response to NPN compounds has been demonstrated have generally involved the use of semi-purified diets or purified amino acid diets. Featherston *et al.* (1961, 1962a), feeding a crystalline amino acid diet, demonstrated significant use of urea and DAC by chicks. They used a basal diet containing only L-essential amino acids, except for DL methionine, at levels to meet NRC requirements. They found that chicks fed iso-nitrogenous diets, in which nitrogen for synthesis of non-essential amino acids was supplied by DAC, urea, or non-essential amino acid mixture, gained significantly more weight than chicks fed the basal diet. These three supplements were equally

effective for growth. However, nitrogen retention studies showed that chicks fed the non-essential amino acid mixture retained a higher percent of ingested nitrogen than chicks fed the other two supplements. Grams of weight gained per gram of nitrogen consumed were 22.0, 20.8 and 19.7 for chicks fed the basal diet supplemented with non-essential amino acids, DAC and urea, respectively. Growth responses of equal magnitude were also observed when nitrogen for non-essential amino acid synthesis was supplied by either excess L-essential amino acids or D-amino acids. They concluded that these various nitrogen supplements were equally effective for chick growth of 4 to 5 g. per day.

Featherston et al. (1962b) conducted another experiment to observe the effectiveness of urea and ammonium nitrogen for the synthesis of dispensable amino acids by chicks. Basal diets with two levels of L-essential amino acids were used in this trial: (1) at NRC requirements and (2) at 1.25 times the NRC requirements. Various levels of urea, DAC and non-essential amino acids were added to the two basals. They found that chicks fed amino acids diets that permitted gains in weight of 4 to 5 g. per day utilized urea and DAC to achieve as rapid growth, although possibly not as efficient, as that achieved by the inclusion of dispensable amino acids in the diet. Feeding urea and DAC increased nitrogen retention and increased plasma levels of non-essential amino acids. When the levels of essential amino acids of the basal diet were increased, urea was not as effective as the intact non-essential amino acids. They concluded that urea and DAC could be utilized by the chicks for non-essential amino acid synthesis when the diets contained a minimum amount

of essential amino acids. Increasing the amounts of essential amino acids in the diet resulted in a decreased utilization of urea and DAC as compared with the use of intact non-essential amino acids in an identical diet.

Olsen et al. (1963) reported reduced weight gains with DAC additions of 8 or 12% to a semi-purified diet containing soybean protein or casein. Scott et al. (1966) also reported that the iso-nitrogenous substitution of DAC for 10% L-glutamic acid in a crystalline amino acid diet reduced chick weight gain by 20%.

Moran et al. (1967) fed a 10% protein, corn-soybean meal basal diet to broiler-type chicks and found that additions of methionine, lysine and glycine to the basal diet caused a significant improvement in chick weight gain. DAC, however, when added to the basal diet at the equivalent of 5% protein, significantly depressed growth, regardless of whether or not essential amino acids were added. When the DAC level was reduced to a 2% protein equivalent, there were no effects on gains. This result was confirmed by the work of Reid (1967). Studies done by Farlin et al. (1968) showed that biuret reduced feed intake and growth when added to a crystalline amino acid diet at levels exceeding 2.3%, while urea at 2.03% improved weight gains and feed efficiency. However, the improvements were not as great as that obtained by adding 10% glutamic acid to the basal diet. Biuret toxicity for broilers was also shown by the work of Berry et al. (1956).

Ammonium acetate and diammonium phosphate (DAP) were also shown to be utilized by chicks. By using carbohydrate-containing diets and

carbohydrate-free diets, Renner (1969) found that ammonium acetate appeared to be utilized as a source of nitrogen for chick growth nearly equal to glutamic acid in carbohydrate-containing diets but not in carbohydrate-free diets. Blair and Waring (1969) found that addition of 1.5% DAP to diets containing essential amino acids at minimum required levels improved broiler growth in one out of two experiments, but higher levels (3.0 or 4.4% DAP) significantly depressed growth of birds in all trials. In their second experiment, they also showed a significant utilization of diammonium sulphate.

In many species of animals, the reversible deamination of glutamic acid is a major mechanism for the interconversion of alpha-amino N and ammonia. This conversion is known to involve glutamic dehydrogenase, which is present in chick liver. Blair and Young (1970) were the first to take advantage of this fact. First they confirmed that alpha-amino N could be converted to glutamic acid by a chick liver system by incubating liver homogenates for 30 minutes with alpha-$^{14}$C-ketoglutaric acid. They found that alpha-ketoglutaric acid could be converted to glutamic acid by chick liver. Secondly, they incubated the liver homogenates with various ammonium salts in the presence and absence of added alpha-ketoglutaric acid. Results showed that in the presence of alpha-ketoglutaric acid there was a good conversion of ammonium nitrogen to glutamic acid, particularly in the case of DAC. The total amount of free amino acids was also increased significantly by the addition of DAC. They summarized that growing chicks were able to utilize ammonium nitrogen. However, the circumstances under which utilization took place

might be somewhat limited. They expected NPN utilization to be maximal when the diet contained a sufficient, but not excess of essential amino acids, and a deficiency of non-essential amino acids. They also postulated that the citrate part of DAC might be converted in the liver by tricarboxylic acid cycle enzymes to alpha-ketoglutaric acid. The alpha-ketoglutaric acid was subsequently converted to glutamic acid by glutamic dehydrogenase and was then available to the bird as such or might be transaminated to other non-essential amino acids. Blair et al. (1972), supplemented purified diets with glycine, glutamic acid, proline or DAC for broiler chicks. They found that slow growth was obtained unless the diet was supplemented with 1% L-proline. Increasing the level of glycine from 1.0 to 1.6% did not result in a marked growth response. Adding 11.07% DAC to the diet gave a significant growth response and a significant increase in the plasma level of amino acids. The utilization of DAC was equivalent to that of an iso-nitrogenous supplement of glutamic acid in one experiment, but was significantly poorer than that of glutamic acid in another.

Other NPN compounds have been tested for their promotion of chick growth by Lee and Blair (1972). They observed that, compared with the growth of chicks fed a semi-purified amino acid diet, the order of body weights at 21 days of age were: 6.61% triammonium citrate (171 g.), 12% glutamic acid (154 g.), 9.23% DAC (131 g.), 4.05% triammonium phosphate plus 12.56% calcium lactate (123 g.), 2.45% urea (118 g.), basal (89 g.) and 17.05% monoammonium citrate (66 g.). In these studies, the NPN compounds were added on an iso-nitrogenous basis. The authors suggested

that the varying responses might be due in part to the differing levels of citrate in the diet. In their second experiment, addition of 3.43% uric acid to basal diet was shown to be less efficient in promoting growth than basal diet.

In 1972, Kazemi and Balloun[1] conducted an experiment using DAC at 2.5, 3.0, 4.5 and 6.0% protein equivalent in 17.5% protein semi-purified diets. They found that DAC, at 2.5 or 3.0% protein equivalent, reduced weight gain slightly. Growth of chicks was severely depressed (up to 50%) at 4.5 or 6.0% protein equivalent. Chicks fed DAC-free rations retained a higher percentage of the ingested nitrogen and had more serum nitrogen compared to chicks fed the low-DAC or high-DAC diets.

Using a cellulose-diluted commercial broiler mash, McNab et al. (1972) were unable to observe an advantage of replacing cellulose with TAC or diammonium hydrogen citrate (DAHC) in a low-protein diet of chicks from 1 to 3 weeks of age.

An experiment on urea tolerance in growing chicks was carried out by March and Bieley (1971). Urea was fed at dietary levels of 1 and 2% to chicks from day-old to 8 weeks of age. The response was tested with four basal diets containing 20, 22, 24 and 26% protein and 2850, 2950, 3050 and 3150 kcal. M.E. per kg., respectively. They found that urea depressed growth of chicks fed 22-24% protein diets, but had no effect on chicks fed the 20% protein diet. Chicks fed 2% urea consumed 20%

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[1]R. S. Kazemi and S. L. Balloun, Poultry Science Department, Kildee Hall, Iowa State University, Ames, Iowa. Personal communication, 1974.

more water than did the controls. Serum uric acid levels were significantly increased when urea was fed and mortality was unaffected by urea at the levels fed.

## Non-protein Nitrogen for Laying Hens

Hall and Helbacka (1959) found that feeding ammonium chloride or hydrochloric acid to layers induced the production of thin shelled eggs. Hunt and Aitken (1962a) used ammonium chloride as a shell quality depressant in their experiment on the effect of ascorbic acid on egg shell quality. They found that $NH_4Cl$ induced production of thin shelled eggs, and could also increase bone ash. Later, Hunt and Aitken (1962b) designed an experiment to investigate the mode of action of $NH_4Cl$ on shell formation, and to establish the dietary level of this salt necessary to induce a change in shell quality. They fed laying hens up to 2% $NH_4Cl$ or other sources of ammonium or chloride ions including HCl, KCl, $CaCl_2$, $(NH_4)_2SO_4$, $(NH_4)_2CO_3$ and $NH_4C_2H_3O_2$ equivalent to 1% $NH_4Cl$. Their results showed that feeding 2% $NH_4Cl$ improved albumen quality while lower levels of this salt were ineffective. Specific gravity of the eggs was markedly affected by 2% $NH_4Cl$ while 1% $NH_4Cl$ and HCl caused significant reduction in egg specific gravity. Two percent $NH_4Cl$ caused a significant decrease in blood pH and plasma bicarbonate and plasma calcium was not influenced by any of the dietary treatments. By applying the concept of Gutowska and Mitchell (1945):

$$2HCO_3^- \rightleftharpoons H_2CO_3 + CO_3^=$$

and $H_2CO_3 \xrightarrow[\text{anhydrase}]{\text{carbonic}} CO_2 + H_2O$ ,

they postulated that the reduction in plasma bicarbonate could limit the availability of carbonate ions for egg shell formation but they failed to explain the action of $NH_4Cl$ on the activity of carbonic anhydrase.

By adding 2% $NH_4Cl$ to a control ration, Hunt (1964) found that $NH_4Cl$ resulted in a decrease (8.5%) in the sodium concentration and an increase (7.7%) in the potassium concentration of albumen. Chloride concentration in albumen increased (53%) significantly while the specific conductance of thin albumen also increased. Albumen and blood pH decreased under $NH_4Cl$ treatment.

Young et al. (1965) were the first to report that 3% protein equivalent from DAC, when added to a 13% protein corn-soybean meal or corn-soybean meal-fishmeal diet, improved egg production up to that obtained with a 16% protein diet. Chavez et al. (1966) conducted two experiments to determine the effect of supplementing laying hen diets with non-protein nitrogen as a partial substitute for the protein. In their experiment, two control diets were used: one formulated to meet the essential amino acid requirements at a minimum protein level, and the other was formulated to meet the essential amino acid requirements at a protein level of 15.75%. They found that egg production and egg size were significantly improved when DAC was fed to supply 3% protein equivalent in the 15.75% protein diet. Both egg size and production were equivalent to those of birds fed a diet containing 15.75% intact protein. Feeding urea at the same level, however, did not improve egg production or egg size. They also compared DAP with fish meal as a source of

protein in a 14.75% protein diet. DAP or fish meal were added displacing cellulose at 2% protein equivalent. Both DAP and fish meal improved egg production and egg size significantly when compared with hens fed a 12.75% protein control diet. They concluded that both DAC and DAP were good sources of NPN for laying hens if care is taken to meet all the essential amino acids. Moran et al. (1967), however, using higher levels of DAC and urea (5%) added to a 10% protein practical diet, or DAC at 2.4 or 6% added to a 16% protein semi-purified diet adequate in essential amino acids but low in non-essential amino acids, failed to improve layer performance. Similar results were reported by Akintunde et al. (1968). In addition, they found that DAP did not improve layer performance.

Shannon et al. (1969) reported that the mean percentages of dietary nitrogen absorbed were 82.9, 85.2 and 85.2 for 10.8% crude protein basal diet, basal plus DAP at 1.8% protein equivalent and basal plus DAC at 1.7% protein equivalent respectively. The percentages of the nitrogen of DAP and DAC absorbed were 97.8 and 99.0, respectively. They concluded that the nitrogen of DAC and DAP was readily absorbed by adult hens, but the amount retained was different. Reid et al. (1971) conducted a series of 3 experiments on the effects of DAP, ammonium sulfate and DAC on layers. They reported that adding DAP to supply 3% protein equivalent in 12.8% dietary protein level significantly decreased egg production. A significant improvement in egg production was obtained when DAP (equivalent to 2% protein) was added to a 13.5%

protein corn-soy diet, but not with 11% protein diet. Diammonium sulfate and DAC also improved egg production when supplemented in a diet containing 13.5% intact protein.

Urea tolerance in laying pullets was tested by March and Bieley (1971). During a 16-week period, urea at dietary levels of 0.5 and 1.0% was fed to layers in a practical laying ration containing 16% protein. They found that mortality, rate of production, egg size, interior quality and shell thickness were unaffected by inclusion of urea in the diet.

In 1972, Kazemi and Balloun[1] found that DAC and urea (at 2.0 and 4.0% protein equivalent) did not improve layer performance. On the contrary, weight gain, egg production, egg weight, feed consumption per hen per day and feed consumption per kg. of eggs were significantly decreased when urea or DAC was added to the diet. When crystalline amino acids were supplemented to the diet, the performance of hens fed DAC and urea rations improved.

Recent studies by Fernandez et al. (1973) indicated that when urea was supplemented to a diet containing 12.5% protein at levels to increase the crude protein of the diet by 1.5 and 3.0%, a graded response in egg production was obtained. A similar supplementation with monosodium glutamate had no effect on egg production. DAC supplementation at 1.5% protein equivalent produced a small increase in egg production, but DAC

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[1]R. S. Kazemi and S. L. Balloun, Poultry Science Department, Kildee Hall, Iowa State University, Ames, Iowa. Personal communication, 1974.

at 3% protein equivalent depressed egg production and feed intake. When the level of protein in the basal diet was reduced to 11.5%, supplementation of DAP to the diet caused a depression in egg production but not in feed intake or nitrogen retention. The results of this experiment confirmed the work of Fernandez and McGinnis (1971).

Blair and Lee (1973) supplemented 1.15% urea to a basal diet containing 11.5% protein, with and without 1.54% added essential amino acids. They reported that egg production, feed intake, feed conversion efficiency, gross efficiency of nitrogen conversion and the ability of hens to maintain body weight were improved by supplementation with essential amino acids, but supplementation with essential amino acids and urea to give the equivalent of 16% protein did not result in significantly higher egg production than that obtained with urea alone. The smallest eggs were produced by hens fed the diet supplemented with urea. The gross composition of eggs was not influenced significantly by dietary treatment but albumen quality (Haugh units) was significantly lower on the basal and higher on the urea diet.

## The Effects of Drug Treatments on NPN Utilization

Since early studies of urea utilization by chicks were found unsatisfactory, Slinger et al. (1952) suggested that an increased utilization of urea might occur when antibiotics were included in the diet. They conducted a series of 3 experiments. Basal diets contained 10.5, 17.0 and 15.5% protein for Experiments 1, 2 and 3, respectively.

Different levels of urea (0.38% to 4%) were added to the rations with and without 10 ppm procaine penicillin G. They found that weight gains of chicks were not improved by the addition of urea to diets sub-optimal in protein level either in the presence or the absence of penicillin in the diet. In the absence of penicillin, the feed:gain ratio tended to be higher at higher levels of urea. However, in the presence of penicillin this effect was not observed. Similar experiments were performed by Jones and Combs (1953). They used aureomycin HCl at levels of 25 ppm in Experiment 1 and 10 ppm in Experiment 2. Basal diets contained 15 and 17% protein for Experiment 1 and 2, respectively, and a 20% protein practical diet was used as a positive control. Their results showed that addition of urea (1%), ammonium citrate (3.9%) or di-ammonium phosphate (2.2%), replacing an equal amount of corn, had little or no effect on growth or feed efficiency in the 17% protein ration, with or without aureomycin HCl. When the 15% protein basal ration was employed, the addition of urea did not affect growth rate but reduced efficiency of feed utilization when no aureomycin HCl was fed. The addition of ammonium citrate or dibasic ammonium phosphate, in the absence of the antibiotic, reduced growth rate as well as feed efficiency. However, in the presence of 10 ppm of aureomycin HCl, the addition of these NPN compounds did not adversely affect either the growth rate or feed efficiency. They concluded that urea, ammonium citrate and dibasic ammonium phosphate could not be utilized by the chicks as a source of nitrogen, even when aureomycin HCl was added at levels of 10 or 25 ppm.

Bare et al. (1963) conducted an experiment on the effect of

antibiotics upon the growth-depressing effect of uric acid in chicks. They fed day-old chicks a uric acid-containing diet, with and without a zinc bacitracin-procaine penicillin mixture. They found that chicks receiving the diet without antibiotics showed 10% weight depression at four weeks, but the depression was not detected in chicks receiving uric acid and antibiotics. Bacteriological analysis of the small intestine contents showed an increase in numbers of uricolytic Aerobactor spp. and an increased degradation of uric acid in the tract of the "uric-antibiotic" chicks. Antibiotic assay showed rapid penicillin inactivation in the tract, but persistance of the bacitracin. The increased uricolysis was observed only in the "uric-antibiotic" chicks.

# EXPERIMENTAL PROCEDURES

## Studies with Laying Hens

One experiment on the effects of DPW upon the performance of laying hens was conducted.

### Experiment I

#### Objectives

This experiment was designed to observe the effects of using 12.5% and 25% DPW in the ration on the performance of laying hens as measured by egg weight, feed per hen per day, egg production, percent hens out of production and mortality. The present study was also undertaken to investigate the possibilities of differing DPW utilization by ages.

#### General procedure

Diets DPW collected from laying hens was used in this experiment. Crude protein content of the DPW was analyzed to be 22.5%. Considering 45% of the crude protein was true protein, three diets were formulated with 3 levels of the DPW: 0, 12.5 and 25% (Table 1). All diets were equivalent in percent true protein, and metabolizable energy levels were kept at approximately 2870 kcal./kg. Calcium and phosphorus levels were increased to 4.01 and 0.86%, respectively, by limestone and defluorinated phosphate. Since chemical analysis of the DPW showed that its phosphorus content was 2.44%, it was not necessary to supply the diet containing 25% DPW with defluorinated phosphate.

Management and data collection This experiment was carried out

Table 1. Ration composition - Experiment I

| Ingredients | Experimental diets (%) 1 | 2 | 3 |
|---|---|---|---|
| Ground yellow corn | 65.0 | 56.0 | 46.4 |
| Soybean meal (48.5% protein) | 20.2 | 19.7 | 19.0 |
| Alfalfa meal (17.0% protein) | 2.0 | 1.1 | 1.0 |
| Defluorinated phosphate | 3.1 | 1.5 | - |
| Ground limestone | 7.7 | 5.5 | 2.9 |
| Vitamin premix[a] | 0.5 | 0.5 | 0.5 |
| Mineral mix[b] | 0.5 | 0.3 | 0.3 |
| Soybean oil | 1.0 | 3.0 | 5.0 |
| DPW | - | 12.5 | 25.0 |
| Calculated analysis: | | | |
| True protein (%) | 16.00 | 16.00 | 16.07 |
| M.E. (kcal./kg.) | 2868 | 2870 | 2864 |
| Calcium (%) | 4.01 | 4.06 | 4.00 |
| Phosphorus (%) | 0.86 | 0.86 | 0.86 |

[a]Supplied per kilogram of diet: vitamin A, 8000 IU; vitamin $D_3$, 1500 ICU; vitamin $B_{12}$, 5 mcg.; riboflavin, 5 mg.; pantothenic acid, 5 mg.; niacin, 15 mg.; choline, 325 mg.; methionine, 500 mg.

[b]Supplied per kilogram of diet: NaCl, 4.6 g.; Mn, 66 mg.; Zn, 25 mg.; Fe, 18 mg.; Cu, 3 mg.; $I_2$, 1.3 mg.; Co, 0.28 mg.

in a windowless cage house with thermostatically controlled ventilating fans. Ninety-six 8 month-old laying hens (Welp-line) were weighed and distributed in 25.4 x 40.6 cm. individual cages. Eight adjacent cages with two common feeders constituted an experimental unit. Extremely heavy or light birds were discarded and all experimental birds were leg-banded for identification. Feed and water were available to the birds ad libitum throughout the five-month test period and the hens had 14 hours of light daily. In order to minimize feed wastage, feeders were covered with wire-mesh screens.

For each of the three treatments, there were four replicate groups with 8 birds per group. The experiment was a randomized complete-block arrangement of treatments in a split-plot design with periods (months) as sub-plots. During the first two weeks of the test period, dead birds were replaced by spares of approximately same weight.

Eggs were collected two times daily for the calculation of monthly percent egg production. After the first month of the test period, all eggs laid on three consecutive days at the end of each month were weighed for average egg weight estimation. Feed consumption of each experimental group was also recorded at the same time. After the second week of the test period, records of mortality were kept without replacing the dead birds. Hens that laid less than 9 eggs per month were considered out of production and the records of these hens were collected for the calculation of monthly percentage of hens out of production.

Statistical analysis of the data were completed according to methods described by Snedecor and Cochran (1967), Cochran and Cox (1968),

Steel and Torrie (1960), Zimmerman[1] and Cox[2].

Results

Data on feed consumption, average egg weight, percent egg production, percent hens out of production and mortality by periods are presented in Table 2. Percent egg production and percent hens out of production are also given graphically in Figures 1 and 2, respectively. Corresponding analysis of variance are given in Appendix A, Table 20. Daily feed consumption of birds decreased linearly ($P_{Lin.} \leq 0.01$) as the level of DPW in the diets increased. Feed consumption per hen per day significantly decreased by periods ($P_{Quad.} \leq 0.05$; $P_{Cub.} \leq 0.01$). A significant ($P<0.01$) dietary treatment x period interaction was observed in daily feed consumption.

The data of egg production were calculated from hens in production and on hen-day basis. Egg production of birds fed the control diet was significantly ($P<0.05$) more than that of birds fed DPW diets in a linear fashion ($P_{Lin.} \leq 0.01$); as DPW level in the diet increased, egg production decreased. A difference of 5.7% egg production was observed between birds fed the control diet and birds fed the 25% DPW diet. Egg production significantly ($P<0.01$) decreased by periods. The decrease was significant ($P_{Lin.} \leq 0.01$). During the second period, birds

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[1]D. R. Zimmerman, 337 Kildee, Iowa State University, Ames, Iowa. Personal communication, 1974.

[2]D. F. Cox, 124 Snedecor, Iowa State University, Ames, Iowa. Personal communication, 1974.

Table 2. The effects of DPW on feed consumption, average egg weight, percent egg production, percent hens out of production and mortality of laying hens - Experiment I[a]

| Dietary treatments | Months | | | | | |
|---|---|---|---|---|---|---|
| | June | July | August | September | October | Mean |
| | | | g. feed/hen/day | | | |
| 0.0% DPW | 102.5 | 102.1 | 97.0 | 98.9 | 106.6 | 101.4 |
| 12.5% DPW | 96.0 | 102.8 | 100.2 | 98.2 | 99.3 | 99.3 |
| 25.0% DPW | 100.8 | 104.0 | 90.6 | 89.1 | 98.5 | 96.6 |
| Mean | 99.8 | 103.0 | 95.9 | 95.4 | 101.5 | 99.1 |
| | | | Average egg weight (g.) | | | |
| 0.0% DPW | - | 57.4 | 57.1 | 60.6 | 61.9 | 59.3 |
| 12.5% DPW | - | 58.5 | 58.8 | 61.7 | 63.9 | 60.7 |
| 25.0% DPW | - | 59.0 | 57.6 | 61.7 | 64.2 | 60.6 |
| Mean | - | 58.3 | 57.8 | 61.3 | 63.3 | 60.2 |
| | | | Percent egg production[b] | | | |
| 0.0% DPW | 78.3 | 80.3 | 74.8 | 70.0 | 69.2 | 74.5 |
| 12.5% DPW | 75.0 | 76.9 | 75.4 | 66.7 | 69.1 | 72.6 |
| 25.0% DPW | 76.7 | 76.8 | 66.6 | 64.5 | 59.4 | 68.8 |
| Mean | 76.7 | 78.0 | 72.3 | 67.1 | 65.9 | 72.0 |
| | | | Percent hens out of production[c] | | | |
| 0.0% DPW | - | 3.1 | 12.5 | 12.5 | 21.9 | 10.0 |
| 12.5% DPW | 6.3 | 9.4 | 6.3 | 6.3 | 29.5 | 11.5 |
| 25.0% DPW | 6.3 | - | 6.3 | 24.1 | 28.6 | 13.0 |
| Mean | 4.2 | 4.2 | 8.4 | 14.4 | 26.8 | 11.6 |
| | | | Mortality (%) | | | |
| 0.0% DPW | | | 0.0 | | | |
| 12.5% DPW | | | 6.3 | | | |
| 25.0% DPW | | | 12.5 | | | |

[a]All values represent means of 4 replicate groups, 8 hens per group.

[b]Calculated from hens on production.

[c]Hens laying less than 9 eggs per month were considered out of production.

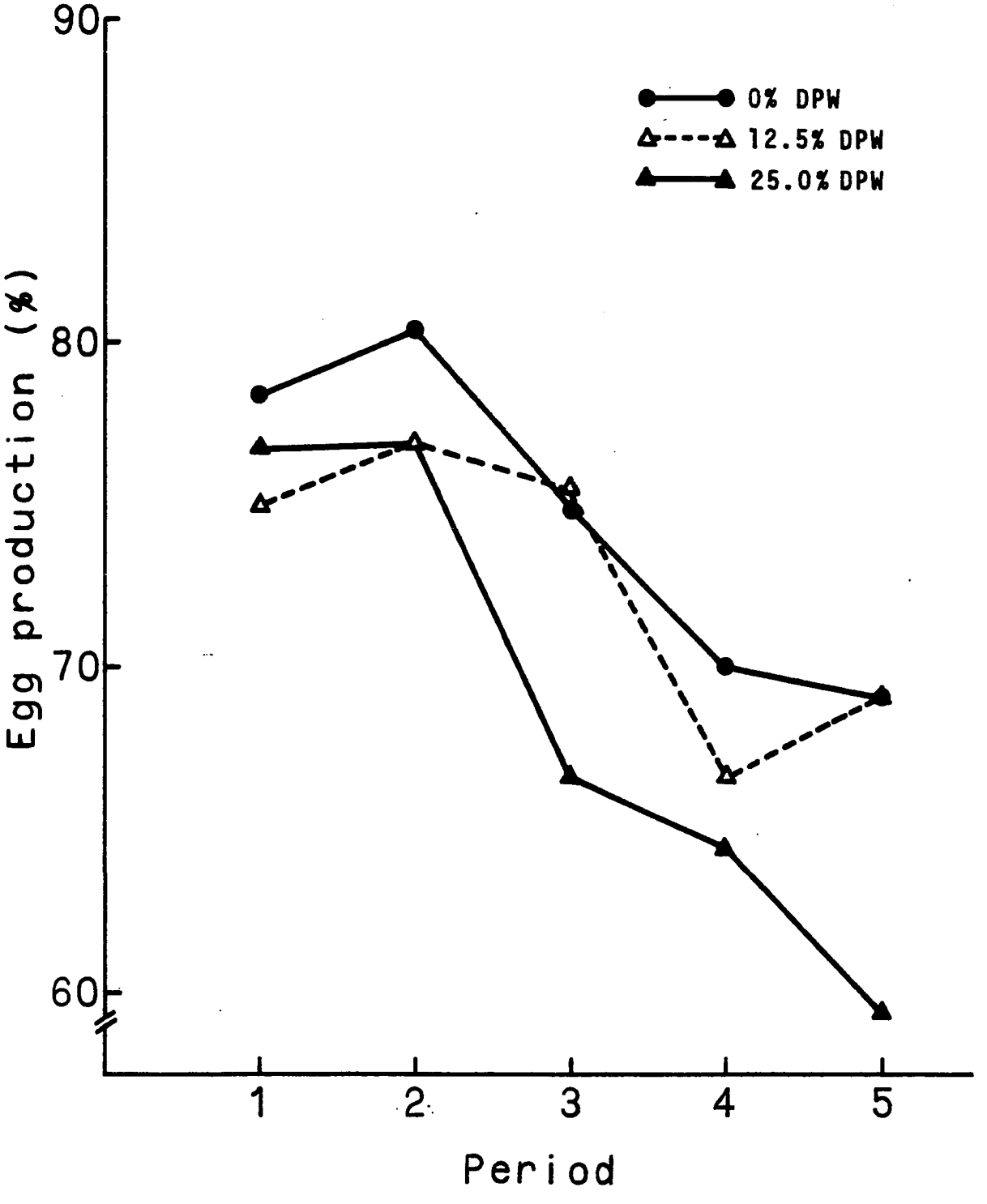


Figure 1. The effects of DPW on egg production by periods - Experiment I

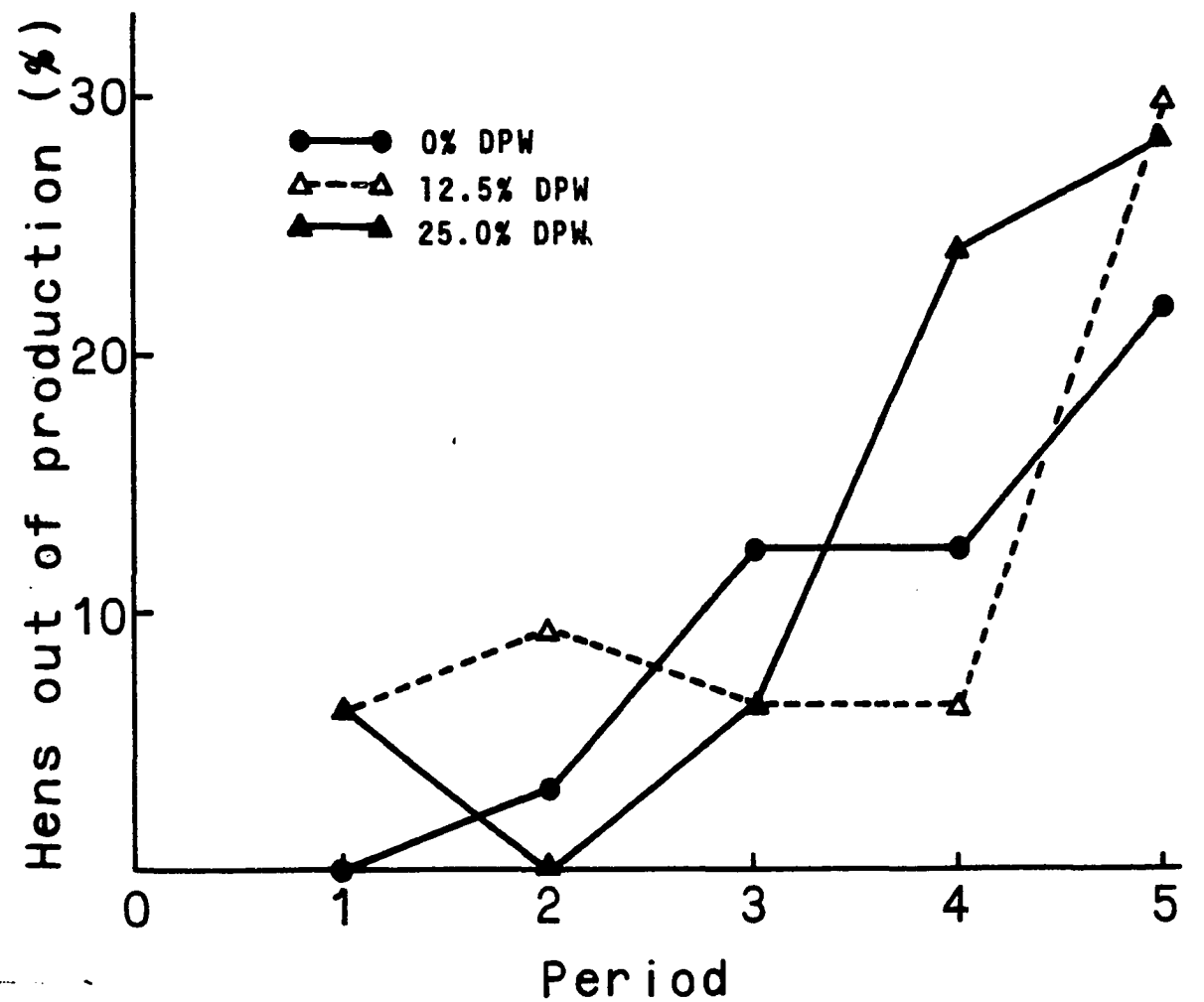


Figure 2. The effects of DPW on percent hens out of production by periods - Experiment I

fed the control and 12.5% DPW diets increased their egg production about 2% (as compared with period 1) but birds fed 25% DPW diet failed to do so.

Considering hens that laid less than 9 eggs per month were out of production, percent hens out of production seemed to increase as the level of DPW in the diet increased. But statistical analysis showed no significant difference among dietary treatments. A significant ($P<0.01$) difference by periods was observed in the percentage of hens out of production. Statistically, the increase in percentage of hens out of production by period was significant in both linear and quadratic manners ($P_{Lin.} \leq 0.01$; $P_{Quad.} \leq 0.05$). During the last two periods (the fourth and fifth periods), percentage of hens out of production of birds fed 25% DPW diet increased drastically.

Egg weight of hens fed the control diet tended to be less than that of birds fed DPW diets but the difference was not statistically significant. In all cases of dietary treatments, the birds laid significantly ($P<0.01$) heavier eggs as they got older. Linear, quadratic and cubic responses were observed to be significant ($P_{Lin.} \leq 0.01$; $P_{Quad.} \leq 0.01$; $P_{Cub.} \leq 0.01$) in the increase of egg weight by periods.

Percent mortality was calculated at the end of the experiment from the total number of birds receiving the same dietary treatment. Dietary level of DPW seemed to affect mortality of birds. There was no mortality of birds fed the control diet but, as the level of DPW in the diet increased from 12.5 to 25%, the percent mortality increased to 6.25 and 12.50%, respectively. However, this apparent linear response might be

accidental.

## Studies with Young Birds

Five experiments on the effects of DPW, NPN, drug treatments and added amino acids upon young chickens were conducted.

### General Pre-experimental Management

Commercial day-old broiler-strain male chicks obtained from Welp's Poultry Farm (Bancroft, Iowa) were used in all experiments. The chicks were distributed in 6-deck electrically heated battery brooders equipped with wire floors. Temperature was regulated by thermostats at approximately $35^{\circ}$, $32.2^{\circ}$, $29.4^{\circ}$ and $26.6^{\circ}$C. during the first, second, third and fourth week of age, respectively. The heat was then turned off.

Water and a standard chick starter ration were available to the chicks ad libitum until experiments were started, at which time feed was administered according to ration formulation as will be discussed in detail later. Lights were provided at all times.

### Experiment II

#### Objectives

This experiment was undertaken to determine the effects of DAC, DPW and added amino acids on the performance, nitrogen utilization and nitrogen gain of broiler-type chicks. The possibilities of increasing nitrogen utilization and nitrogen gain by chicks with age were also studied.

#### General procedure

Diets In this experiment, there were 11 experimental diets

(Tables 3 and 4). Test diets were formulated so that all diets were isocaloric (3004 M.E. per kg.) with various levels of DPW (10, 20%), and DAC[1] (2.85, 5.7%). The DPW used in this experiment was collected from laying hens kept in cages as wet excreta, and was dried in an air-blow oven, equipped with thermostatically-controlled electric heating units, at 80 ± 2°C. for 72 hours. The dried product was then ground before mixing in the diets. Two basal diets were used as control rations: high-protein control (19.32%) and low-protein control (15.11%). DPW and DAC were added to the low-protein basal diet with some changes in dextrose, cellulose and soybean oil contents to obtain isocaloric diets. The added essential amino acids were just adequate to meet NRC (1966) requirements. Diets were formulated on crude protein basis instead of true protein basis as in Experiment I. Since chemical analysis of the DPW showed that its calcium and phosphorus contents were 5.5% and 2.56%, respectively, it was not necessary to supply the diet containing 20% DPW with defluorinated phosphate and limestone.

The purified ration shown in Table 4 was formulated so that it was nitrogen-free but met NRC (1966) requirements for all other nutrients. This diet was formulated to be high in cellulose content (17.32%) for the purpose of increasing the amount of excreta.

Management and data collection The experiment was carried out in a windowless battery room with central-thermostatically controlled

[1]Diammonium citrate, Baker Chemical Company (12.39% nitrogen).

Table 3. Ration composition - Experiment II

| Ingredients | Experimental diets (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Diammonium citrate | - | - | - | - | - | - | 2.85 | 2.85 | 5.70 | 5.70 |
| Dried poultry waste (DPW) | - | - | 10.00 | 10.00 | 20.00 | 20.00 | - | - | - | - |
| Ground yellow corn | 64.07 | 41.32 | 41.32 | 41.32 | 41.32 | 41.32 | 41.32 | 41.32 | 41.32 | 41.32 |
| Soybean meal (48.5% protein) | 27.39 | 23.01 | 23.01 | 23.01 | 23.01 | 23.01 | 23.01 | 23.01 | 23.01 | 23.01 |
| Soybean oil | 0.95 | 2.23 | 2.23 | 2.23 | 5.60 | 5.60 | 2.23 | 2.23 | 5.60 | 5.60 |
| Cellulose | 1.90 | 5.78 | 0.81 | 0.81 | - | - | 2.96 | 2.96 | 4.29 | 4.29 |
| Dextrose | - | 21.78 | 18.73 | 18.73 | 8.10 | 8.10 | 21.78 | 21.78 | 14.12 | 14.12 |
| Vitamin premix[a] | 0.49 | 0.49 | 0.49 | 0.49 | 0.49 | 0.49 | 0.49 | 0.49 | 0.49 | 0.49 |
| Salt & mineral mix[b] | 0.49 | 0.49 | 0.49 | 0.49 | 0.49 | 0.49 | 0.49 | 0.49 | 0.49 | 0.49 |
| Defluorinated phosphate | 2.24 | 2.73 | 1.37 | 1.37 | - | - | 2.73 | 2.73 | 2.73 | 2.73 |
| Ground limestone | 1.41 | 1.14 | 0.57 | 0.57 | - | - | 1.14 | 1.14 | 1.14 | 1.14 |
| Lysine | - | - | - | 0.10 | - | 0.15 | - | 0.15 | - | 0.24 |
| Methionine | - | - | - | - | - | - | - | 0.12 | - | 0.24 |
| Arginine | - | - | - | - | - | 0.19 | - | 0.05 | - | 0.29 |
| $Cr_2O_3$ | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |

[a]Supplied per kilogram of diet: vitamin A, 7500 IU; vitamin $D_3$, 1000 ICU; vitamin E, 10 IU; menadione, 2.2 mg.; vitamin $B_{12}$, 10 mcg.; riboflavin, 5 mg.; choline, 450 mg.; pantothenic acid, 10 mg.; niacin, 25 mg.; methionine, 1000 mg.; ethoxyquin, 100 mg.

[b]Supplied per kilogram of diet: NaCl, 4.4 g.; Mn, 117 mg.; Zn, 50 mg.; Fe, 35 mg.; Cu, 6 mg.; $I_2$, 2 mg.; Co, 0.55 mg.

Table 3. (Continued)

| Ingredients | Experimental diets (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Calculated analysis: | | | | | | | | | | |
| Crude protein (%) | 19.32 | 15.11 | 17.22 | 17.32 | 19.32 | 19.66 | 17.22 | 17.54 | 19.32 | 21.09 |
| Fat (%) | 4.24 | 4.07 | 4.28 | 4.28 | 8.04 | 8.04 | 4.07 | 4.07 | 7.62 | 7.62 |
| Calcium (%) | 1.17 | 1.17 | 1.17 | 1.17 | 1.17 | 1.17 | 1.17 | 1.17 | 1.17 | 1.17 |
| Phosphorus (%) | 0.77 | 0.77 | 0.77 | 0.77 | 0.77 | 0.77 | 0.77 | 0.77 | 0.77 | 0.77 |
| Lysine (%) | 1.06 | 0.85 | 0.87 | 0.97 | 0.92 | 1.06 | 0.82 | 0.97 | 0.82 | 1.06 |
| Methionine + cystine (%) | 0.82 | 0.58 | 0.70 | 0.70 | 0.82 | 0.82 | 0.58 | 0.70 | 0.58 | 0.82 |
| Arginine (%) | 1.38 | 1.09 | 1.14 | 1.14 | 1.18 | 1.38 | 1.09 | 1.14 | 1.09 | 1.38 |
| M.E. (kcal./kg.) | 3004 | 3004 | 3004 | 3004 | 3004 | 3004 | 3004 | 3004 | 3004 | 3004 |
| Fiber (%) | 4.33 | 7.58 | 3.93 | 3.93 | 4.48 | 4.48 | 4.73 | 4.73 | 6.07 | 6.07 |

Table 4. Nitrogen-free diet composition - Experiment II

| Ingredients | Percent |
|---|---|
| Dextrose | 72.78 |
| Soybean oil | 4.50 |
| Salt & mineral mix[a] | 4.02 |
| Vitamin mix[b] | 0.38 |
| Cellulose | 17.32 |
| $Cr_2O_3$ | 1.00 |
| Calculated analysis: | |
| Protein (%) | 0.0 |
| M.E. (kcal./kg.) | 3004.6 |
| Calcium (%) | 1.0 |
| Phosphorus (%) | 0.5 |

[a]Supplied per kilogram of diet: Ca, 10 g.; P, 5 g.; Na, 1.5 g.; K, 4 g.; Cl, 1.5 g.; Mn, 55.115 mg.; Mg, 0.551 g.; Fe, 88.2 mg.; Cu, 11.02 mg.; Zn, 44.1 mg.; Se, 150 mcg.; $I_2$, 379 mcg.; Co, 76 mcg.

[b]Supplied per kilogram of diet: vitamin A, 16534 IU; vitamin $D_3$, 1102.3 ICU; vitamin E, 11.02 IU; menadione, 2.2 mg.; thiamine, 2.2 mg.; riboflavin, 4.96 mg.; pantothenic acid, 14.33 mg.; niacin, 33.1 mg.; pyridoxine, 4.4 mg.; biotin, 0.13 mg.; folacin, 1.32 mg.; choline, 1323 mg.; vitamin $B_{12}$, 11.02 mcg.; linoleic acid, 12 g.; ethoxyquin, 99.21 mg.

ventilation and temperature. Four hundred one-week-old male chicks were divided into 2 equal groups and randomly assigned to four 6-deck batteries, except that the bottom decks were not used. The first group of chicks was used to determine nitrogen utilization and nitrogen gain. For this group of chicks, the arrangement of treatments was a randomized complete-block in a split-plot design with batteries as blocks and periods (weeks) as sub-plots. There were 4 replicate groups, 5 birds per group for each dietary treatment. The second group of chicks was used for growth rate and feed efficiency studies. The treatment arrangement of this group was similar to that of the first group except that there was no sub-plot (period).

Feed and water were available to the birds ad libitum throughout the four-week test period and lights were on at all times. For the first group, within each week, chicks were fed the nitrogen-free diet for 3 consecutive days, then the diet was changed to test diets for 4 consecutive days. Excreta were collected in 5% boric acid one day before changing diets by placing a petri dish under each pen. The composite excreta sample was then dried in a thermostatically controlled oven at 80°C. for 48 hours. After the dried excreta samples had been ground through a 40-mesh sieve, they were then assayed for chromic oxide (Bolin et al., 1952) and nitrogen by micro-Kjeldahl method. Chromic oxide and nitrogen in dried feed samples were also determined by the same methods. Percentage of nitrogen utilization was calculated by using the formula shown below. This formula was modified from the formula used

for calculation of biological value. The reason for this modification was that true metabolic nitrogen could not be determined in chickens without separation of urinary nitrogen.

$$\frac{\text{g. nitrogen utilized}}{\text{100 g. intake nitrogen}} = 100\frac{A - \frac{BC}{D} + \frac{EF}{G}}{A}$$

Percent nitrogen gain was calculated by using the formula:

$$\frac{\text{g. nitrogen gained}}{\text{100 g. intake nitrogen}} = 100\,\frac{A - \frac{BC}{D}}{A}$$

where A = g. nitrogen/g. dried diet

B = g. nitrogen/g. dried excreta of bird fed test diet

C = mg. $Cr_2O_3$/g. dried diet

D = mg. $Cr_2O_3$/g. dried excreta of bird fed test diet

E = g. nitrogen/g. dried excreta of bird fed nitrogen-free diet

F = mg. $Cr_2O_3$/g. dried nitrogen-free diet

G = mg. $Cr_2O_3$/g. dried excreta of bird fed nitrogen-free diet

For the second group of chicks, the birds were fed test diets ad libitum throughout the test period. Initial and final weights of feed and birds were taken for the calculation of feed efficiency and weight gain.

Statistical analysis of the data were completed in the same manner as described for Experiment I.

Results

Data for weight gain and feed efficiency are listed in Table 5 and corresponding analysis of variance are given in Appendix A, Table 21. Differences among weight gains and feed efficiencies of birds fed experimental diets were significant ($P<0.01$). Without added amino acids, a difference in weight gain of 42.8 g. between birds fed the low-protein control diet and those fed DPW diets, and 175.5 g. between birds fed the control and birds fed DAC diets were obtained. Feed efficiency of the control birds was also better than that of birds fed DPW or DAC diets. Birds fed diets supplemented with 10% DPW seemed to grow better than the control birds but their feed efficiencies were similar. In contrast, a diet containing 2.87% DAC, and not supplemented with amino acids, lowered weight gain and feed efficiency drastically. At dietary levels of 20% DPW or 5.70% DAC, growth rate and feed efficiency were greatly depressed. As the levels of DPW in diets increased, significant linear and quadratic trends of decreasing weight gain were observed ($P_{Lin.} \leq 0.01$; $P_{Quad.} \leq 0.01$). Feed efficiencies seemed to decrease linearly rather than quadratically ($P_{Lin.} \leq 0.01$; $P_{Quad.} \leq 0.05$). Weight gain and feed efficiency depressions of birds fed increasing levels of DAC, without amino acid supplementation, were linearly significant ($P_{Lin.} \leq 0.01$).

With amino acids added, both weight gain and feed efficiency were greatly improved ($P<0.01$). Similar weight gains were obtained by birds

Table 5. Mean[a] weight gain and feed/gain during 1-5 weeks of age - Experiment II

| Dietary treatments | | Weight gain (g.) | g. feed/g. gain |
|---|---|---|---|
| Basal diet I (19.32% crude protein) | | 724.0 | 2.05 |
| Basal diet II (15.11% crude protein) | | 730.7 | 2.33 |
| Amino acid[b] | DPW (%) | | |
| 0 | +10 | 743.9 | 2.33 |
| | +20 | 631.8 | 2.51 |
| | Mean | 687.9 | 2.42 |
| + | +10 | 765.7 | 2.36 |
| | +20 | 769.1 | 2.17 |
| | Mean | 767.4 | 2.27 |
| Overall mean | | 727.7 | 2.35 |
| Amino acid | DAC (%) | | |
| 0 | +2.87 | 587.6 | 2.52 |
| | +5.70 | 522.8 | 2.57 |
| | Mean | 555.2 | 2.55 |
| + | +2.87 | 722.0 | 2.19 |
| | +5.70 | 739.0 | 2.13 |
| | Mean | 730.5 | 2.16 |
| Overall mean | | 642.9 | 2.36 |

[a]All values represent means of 4 replicate groups, 5 birds per group.

[b]See Table 3, page 40.

fed the low-protein control diet without amino acids added and those fed amino acid-supplemented DAC diets but amino acid-supplemented DPW diets gave better weight gain than the control or DAC diets. On the contrary, the best feed efficiency was obtained from birds fed amino acid-supplemented DAC diets. With amino acids supplemented, performance of birds fed high levels of DAC (5.70%) or DPW (20.0%) was better than that of birds fed the lower levels.

Data for nitrogen utilization and nitrogen gain by periods are shown in Tables 6 and 7, and graphically in Figures 3 and 4. The corresponding analyses of variance are presented in Appendix A, Table 22. In this experiment, the effects of dietary treatments on nitrogen utilization and nitrogen gain were highly significant ($P<0.01$). As the dietary levels of DAC or DPW increased, percentage of nitrogen intake being utilized and gained by birds significantly ($P<0.01$) decreased. A significant ($P<0.01$) linear trend was observed for nitrogen utilization when increasing DPW levels were fed to the birds, and nitrogen gain showed both linear and quadratic responses ($P_{Lin.} \leq 0.01$; $P_{Quad.} \leq 0.05$). When increasing levels of DAC in the diets were fed to the birds, decreasing percent nitrogen utilized and percent nitrogen gained by the birds were observed to be both linear and quadratic. With amino acids supplemented, nitrogen utilization and nitrogen gain of birds fed DAC or DPW diets were greatly improved ($P<0.01$), but they were still lower than those of birds fed control diets without amino acids supplemented. Nitrogen in DPW diets was found to be utilized and gained by the chicks better than that in DAC diets ($P<0.01$). Percentages of nitrogen intake

Table 6. The effects of DAC and added amino acids on nitrogen utilization and nitrogen gain - Experiment II

| Dietary treatments | | Age (weeks) 2 | 3 | 4 | 5 | Mean |
|---|---|---|---|---|---|---|
| | | Percent[a] nitrogen utilized | | | | |
| Basal diet I (19.32% crude protein) | | 69.33 | 70.40 | 74.77 | 73.67 | 72.04 |
| Basal diet II (15.11% crude protein) | | 80.16 | 76.67 | 76.26 | 80.35 | 78.36 |
| Amino acid[b] | DAC (%) | | | | | |
| 0 | +2.85 | 61.68 | 65.66 | 68.50 | 56.50 | 63.09 |
| | +5.70 | 57.44 | 60.00 | 66.18 | 61.78 | 61.35 |
| | Mean | 59.56 | 62.83 | 67.34 | 59.14 | 62.22 |
| + | +2.85 | 68.64 | 71.63 | 75.50 | 78.60 | 73.59 |
| | +5.70 | 58.96 | 60.96 | 67.69 | 69.92 | 64.38 |
| | Mean | 63.80 | 66.30 | 71.60 | 74.26 | 68.99 |
| Overall mean | | 61.68 | 64.57 | 69.47 | 66.70 | 65.61 |
| | | Percent[a] nitrogen gained | | | | |
| Basal diet I | | 60.89 | 57.57 | 59.33 | 54.21 | 58.00 |
| Basal diet II | | 68.20 | 61.10 | 60.12 | 57.48 | 61.73 |
| Amino acid | DAC (%) | | | | | |
| 0 | +2.85 | 51.98 | 49.37 | 48.94 | 41.52 | 47.95 |
| | +5.70 | 47.81 | 48.51 | 49.80 | 44.22 | 47.59 |
| | Mean | 49.90 | 48.94 | 49.37 | 42.82 | 47.77 |
| + | +2.85 | 59.47 | 56.73 | 57.32 | 51.41 | 56.23 |
| | +5.70 | 48.60 | 49.14 | 52.66 | 50.99 | 50.35 |
| | Mean | 54.04 | 52.94 | 54.99 | 51.20 | 53.29 |
| Overall mean | | 51.97 | 50.94 | 52.18 | 47.04 | 50.53 |

[a]All values represent means of 4 replicate groups, 5 birds per group.

[b]See Table 3, page 40.

Table 7. The effects of DPW and added amino acids on nitrogen utilization and nitrogen gain - Experiment II

| Dietary treatments | | Age (weeks) 2 | 3 | 4 | 5 | Mean |
|---|---|---|---|---|---|---|
| | | Percent[a] nitrogen utilized | | | | |
| Basal diet I (19.32% crude protein) | | 69.33 | 70.40 | 74.77 | 73.67 | 72.04 |
| Basal diet II (15.11% crude protein) | | 80.16 | 76.67 | 76.26 | 80.35 | 78.36 |
| Amino acid[b] | DPW (%) | | | | | |
| 0 | +10 | 71.70 | 70.97 | 74.33 | 64.79 | 70.45 |
| | +20 | 63.35 | 67.80 | 77.94 | 65.51 | 68.65 |
| | Mean | 67.53 | 69.39 | 76.14 | 65.15 | 69.55 |
| + | +10 | 71.03 | 66.83 | 79.39 | 69.46 | 71.68 |
| | +20 | 64.78 | 66.77 | 76.92 | 64.75 | 68.31 |
| | Mean | 67.91 | 66.80 | 78.16 | 67.11 | 70.00 |
| Overall mean | | 67.72 | 68.10 | 77.15 | 66.13 | 69.78 |
| | | Percent[a] nitrogen gained | | | | |
| Basal diet I | | 60.89 | 57.57 | 59.33 | 54.21 | 58.00 |
| Basal diet II | | 68.20 | 61.10 | 60.12 | 57.48 | 61.73 |
| Amino acid | DPW (%) | | | | | |
| 0 | +10 | 59.37 | 55.12 | 59.44 | 48.23 | 55.54 |
| | +20 | 54.21 | 54.55 | 63.85 | 47.77 | 55.10 |
| | Mean | 56.79 | 54.84 | 61.65 | 48.00 | 55.32 |
| + | +10 | 60.67 | 53.59 | 66.25 | 52.30 | 58.20 |
| | +20 | 52.96 | 55.37 | 68.10 | 54.32 | 57.69 |
| | Mean | 56.82 | 54.48 | 67.18 | 53.31 | 57.95 |
| Overall mean | | 56.81 | 54.66 | 64.42 | 50.66 | 56.64 |

[a]All values represent means of 4 replicate groups, 5 birds per group.

[b]See Table 3, page 40.

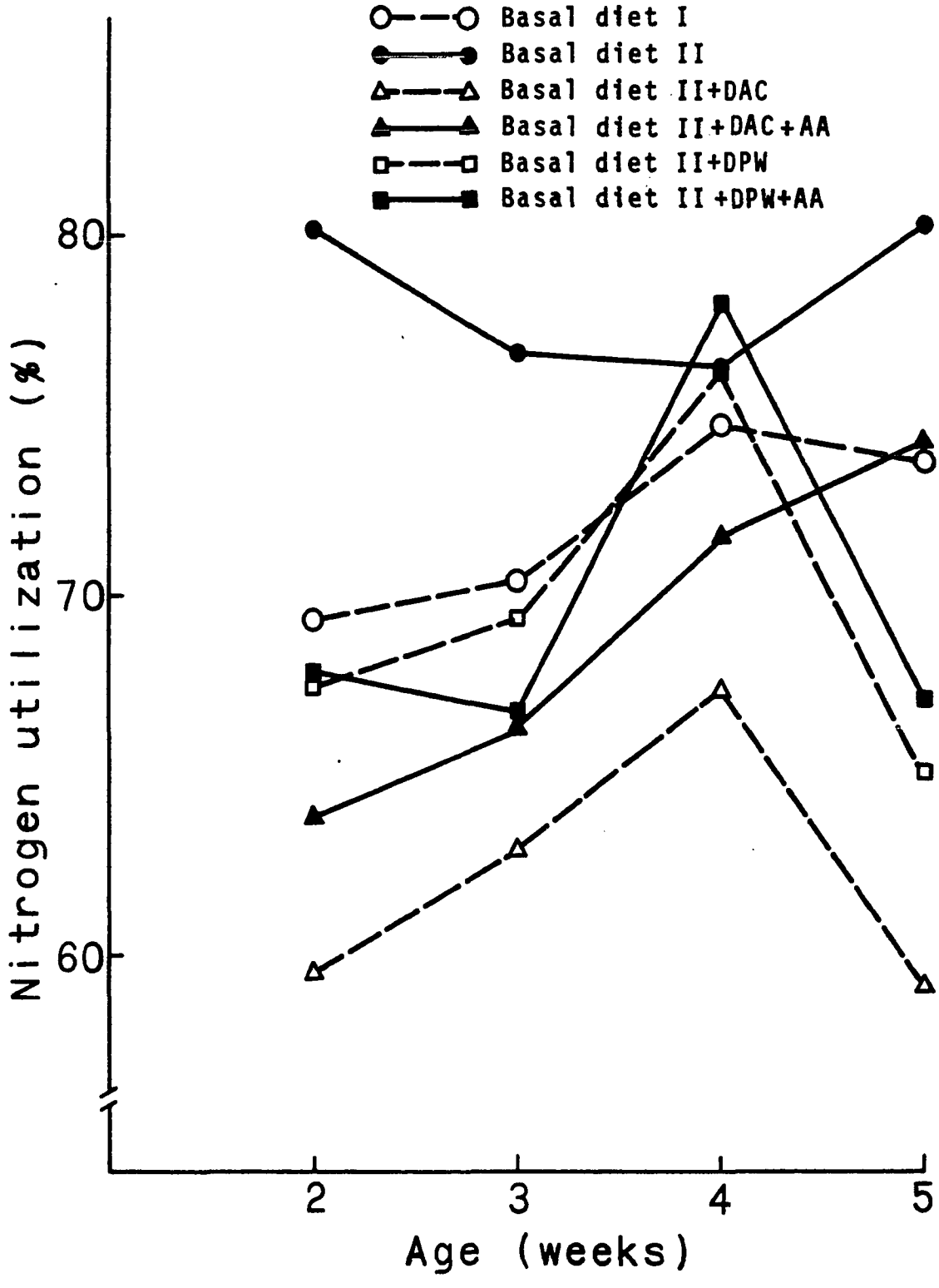


Figure 3. The effects of DAC, DPW and added amino acids on percent nitrogen utilization by periods - Experiment II

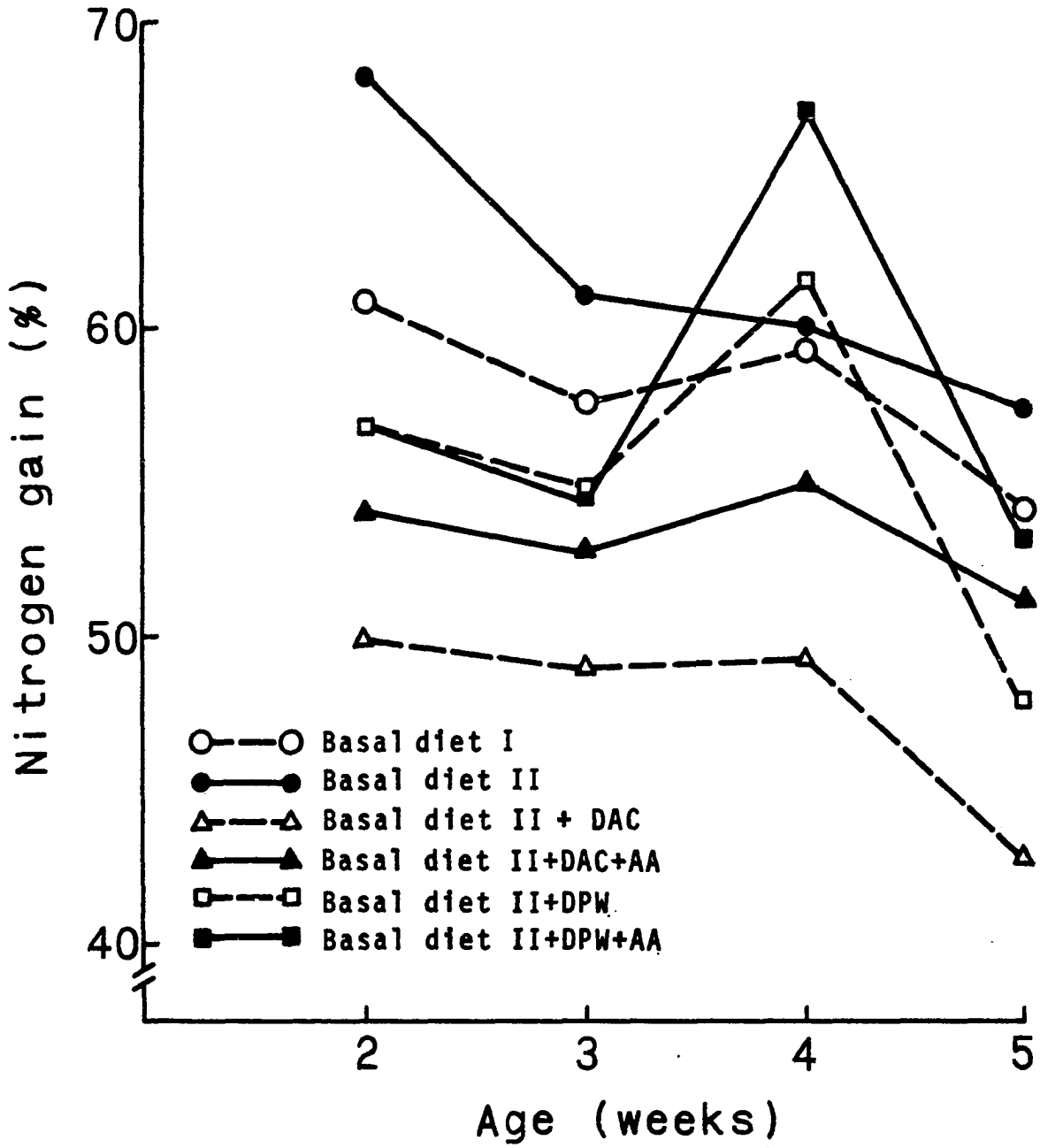


Figure 4. The effects of DAC, DPW and added amino acids on percent nitrogen gain by periods - Experiment II

utilized and gained by birds fed the low-protein control diet (15.11% crude protein) were higher than those utilized and gained by birds fed the high-protein control diet (19.32% crude protein).

Age of birds significantly ($P<0.01$) influenced nitrogen utilization and nitrogen gain of birds fed experimental diets. Linear, quadratic and cubic trends of the influences were observed to be significant ($P_{Lin.} \leq 0.01$; $P_{Quad.} \leq 0.01$; $P_{Cub.} \leq 0.01$). The greatest percentage of nitrogen intake utilized was obtained when the birds were four weeks old, except that birds fed the low-protein control diet and the amino acid-supplemented DAC diets had highest percent nitrogen utilization at five weeks of age. Percentage of nitrogen intake utilized by birds fed the low-protein basal diet was high at two weeks of age and then dropped until four weeks of age, at which time it increased again until the end of the experiment at five weeks of age. For birds fed DAC diets, added amino acids greatly improved percent nitrogen utilization. The improvement was greatest after the birds had been fed the amino acid-supplemented DAC diet for four weeks. In contrast, supplemented amino acids did not greatly improve the nitrogen utilization of birds fed DPW diets. A small improvement was observed during the fourth and fifth weeks. Percentage of nitrogen intake gained by the birds significantly ($P_{Lin.} \leq 0.01$; $P_{Quad.} \leq 0.01$; $P_{Cub.} \leq 0.01$) decreased by weeks. Birds fed DAC and DPW diets seemed to improve their nitrogen gain after the diets were fed to the birds for 2-3 weeks and then nitrogen gain were drastically depressed. Supplemented amino acids greatly improved nitrogen gain of birds fed DAC and DPW diets

during the fourth and fifth weeks. Percentage of nitrogen intake gained by birds fed the low-protein basal diet decreased each week.

## Experiment III

### Objectives

This experiment was conducted to study the influence of DAC and added amino acids on the performance of broiler-type chickens from one to five weeks of age. The possibility of interaction between DAC and added amino acids was also explored.

### General procedure

Diets Six experimental diets were formulated so that all diets were nearly isocaloric (3000 M.E. per kg.) with DAC levels of 0, 2.85 and 5.70% (Table 8). DAC was added to the diets replacing cellulose. Each diet was fed with and without amino acids added. Lysine, methionine and arginine were the only three amino acids added to the diets to meet NRC (1966) requirements. Since ground yellow corn content in all diets was low, dextrose was used to increase the energy level.

Management and data collection The experiment was carried out in the same battery room as Experiment II and the management was similar. The experimental design of this experiment was a 3 x 2 factorial arrangement of treatments in randomized complete-block design. For each dietary treatment, there were 4 replicate groups and 6 birds per group. Feed and water were available ad libitum throughout the test period. Group weights and feed consumption by group were recorded bi-weekly: at 3 and 5 weeks of age.

Table 8. Ration composition - Experiment III

| Ingredients | Experimental diets (%) | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| DAC | - | - | 2.85 | 2.85 | 5.70 | 5.70 |
| Ground yellow corn | 41.75 | 41.75 | 41.75 | 41.75 | 41.75 | 41.75 |
| Soybean meal (48.5% protein) | 23.25 | 23.25 | 23.25 | 23.25 | 23.25 | 23.25 |
| Soybean oil | 2.25 | 2.25 | 2.25 | 2.25 | 2.25 | 2.25 |
| Cellulose | 5.84 | 5.84 | 2.99 | 2.99 | 0.14 | 0.14 |
| Dextrose | 22.00 | 22.00 | 22.00 | 22.00 | 22.00 | 22.00 |
| Vitamin premix[a] | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Salt & mineral mix[a] | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Dicalcium phosphate | 2.76 | 2.76 | 2.76 | 2.76 | 2.76 | 2.76 |
| Ground limestone | 1.15 | 1.15 | 1.15 | 1.15 | 1.15 | 1.15 |
| Lysine (80%) | - | 0.148 | - | 0.148 | - | 0.148 |
| Methionine | - | 0.118 | - | 0.118 | - | 0.118 |
| Arginine | - | 0.046 | - | 0.046 | - | 0.046 |
| Calculated analysis: | | | | | | |
| Crude protein (%) | 15.11 | 15.37 | 17.22 | 17.48 | 19.32 | 19.57 |
| Fat (%) | 4.07 | 4.06 | 4.07 | 4.06 | 7.62 | 7.60 |
| Calcium (%) | 1.17 | 1.17 | 1.17 | 1.17 | 1.17 | 1.17 |
| Phosphorus (%) | 0.77 | 0.77 | 0.77 | 0.77 | 0.77 | 0.77 |
| M.E. (kcal./kg.) | 3004 | 2995 | 3004 | 2995 | 3004 | 2995 |
| Lysine (%) | 0.82 | 0.97 | 0.82 | 0.97 | 0.82 | 0.97 |
| Methionine + cystine (%) | 0.58 | 0.70 | 0.58 | 0.70 | 0.58 | 0.70 |
| Arginine (%) | 1.09 | 1.14 | 1.09 | 1.14 | 1.09 | 1.14 |
| Fiber (%) | 7.58 | 7.56 | 4.73 | 4.72 | 1.88 | 1.87 |

[a]See footnotes a and b, Table 3, page 40.

Statistical analysis of the data were completed in the same manner as in Experiment I.

Results

Data for weight gain and feed efficiency of birds during 1-3 and 3-5 weeks are presented in Table 9, and the corresponding analysis of variance in Appendix A, Table 23. It was found that dietary treatments significantly ($P<0.01$) influenced weight gain and feed efficiency of birds during both periods. For the period of 1 to 3 weeks of age, the diet containing 2.85% DAC without amino acids supplemented was superior to the basal diet, but 5.70% DAC in the diet greatly depressed growth and feed efficiency. When amino acids were supplemented to the diets, growth rate and feed efficiency of birds were significantly ($P<0.01$) improved, especially with the birds fed the basal diet supplemented with amino acids. Without amino acids supplemented, increasing the level of DAC in the diets seemed to cause a quadratic reduction in weight gain and feed efficiency of birds during 1 to 3 weeks of age. With amino acids added, increasing the level of DAC in the diets seemed to cause linear reduction in weight gain and feed efficiency.

During the period 3 to 5 weeks of age, supplemented amino acids and levels of DAC in the diets influenced weight gain of birds in the same manner as the previous period (1 to 3 weeks), but DAC levels in the diets did not significantly influence feed efficiency of the birds during this period. However, increasing the level of DAC in the diet did show a slight quadratic response ($P<0.05$) of feed efficiency.

Table 9. The effects of DAC and added amino acids on weight gain and feed per gain of broilers 1-5 weeks of age - Experiment III

| Dietary treatments | Age 1-3 weeks Amino acids[a] 0 | + | Mean | 3-5 weeks 0 | + | Mean |
|---|---|---|---|---|---|---|
| | | | g. gain/bird[b] | | | |
| DAC (%) | | | | | | |
| 0.00 | 233.5 | 267.0 | 250.3 | 357.0 | 438.9 | 398.0 |
| 2.85 | 250.4 | 252.5 | 251.5 | 399.7 | 417.7 | 408.7 |
| 5.70 | 199.2 | 223.8 | 211.5 | 336.5 | 360.2 | 348.3 |
| Mean | 227.7 | 247.8 | 237.7 | 364.4 | 405.6 | 385.0 |
| | | | g. feed/g. gain[b] | | | |
| DAC (%) | | | | | | |
| 0.00 | 2.11 | 1.99 | 2.05 | 2.51 | 2.27 | 2.39 |
| 2.85 | 2.06 | 1.99 | 2.03 | 2.41 | 2.25 | 2.33 |
| 5.70 | 2.23 | 2.04 | 2.14 | 2.48 | 2.31 | 2.40 |
| Mean | 2.13 | 2.01 | 2.07 | 2.47 | 2.28 | 2.37 |

[a]See Table 8, page 54.

[b]All values represent means of 4 replicate groups, 6 birds per group.

The supplemented amino acids x dietary DAC levels interaction was found to be significant ($P<0.05$) for weight gain of the birds during both periods. No significant interaction was observed for feed efficiency.

## Experiment IV

### Objectives

Since an ultimate goal in poultry production is a closed circuit system in which the only by-product of the system is meat or eggs, this experiment was designed to test the concept of re-cycling the excreta within a closed system for broiler production. The effects of re-cycling on the minerals and 3-nitro-4-hydroxyphenylarsonic acid contents of excreta were also determined. This experiment was also designed for the studies of urea utilization.

### General procedure

Diets There were 5 experimental diets (Table 10) in this experiment. All diets were formulated to be isocaloric (2950 kcal. M.E. per kg.) and were equal in percent true protein (16%). DPW was added to the diets, at 10 and 20% levels, replacing ground yellow corn and alfalfa meal. Soybean oil was used only in the diet containing 20% DPW in order to equalize the energy level and to reduce dust, since DPW was very dusty. Urea was added to the basal diet at 0.43 and 0.86%. $Cr_2O_3$ was added to all diets at 0.3% as an index substance for the determination of percent 3-nitro-4-hydroxyphenylarsonic acid retained by the chicken.

Table 10. Ration composition - Experiment IV

| Ingredients | Experimental diets[a] (%) | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| Ground yellow corn | 70.1 | 66.0 | 60.7 | 70.1 | 70.1 |
| Soybean meal (48.5% protein) | 16.2 | 16.6 | 16.3 | 16.2 | 16.2 |
| Alfalfa meal (17.0% protein) | 8.0 | 4.0 | 2.0 | 8.0 | 8.0 |
| Dicalcium phosphate | 2.4 | 1.2 | - | 2.4 | 2.4 |
| Ground limestone | 2.3 | 1.2 | - | 2.3 | 2.3 |
| Vitamin premix[b] | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Mineral mix[b] | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Soybean oil | - | - | 0.4 | - | - |
| DPW | - | 10.0 | 20.0 | - | - |
| Urea | - | - | - | 0.43 | 0.86 |
| $Cr_2O_3$ | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| Calculated analysis: | | | | | |
| Approximate true protein (%) | 16.0 | 16.0 | 16.0 | 16.0 | 16.0 |
| Nitrogen (%) | 2.56 | 2.76 | 2.96 | 2.76 | 2.96 |
| M.E. (kcal./kg.) | 2950 | 2954 | 2953 | 2950 | 2950 |
| Calcium (%) | 1.64 | 1.63 | 1.63 | 1.64 | 1.64 |
| Phosphorus (%) | 0.77 | 0.78 | 0.78 | 0.77 | 0.77 |
| Fiber (%) | 3.84 | 4.32 | 5.15 | 3.84 | 3.84 |

[a]Each diet contains 50 ppm of 3-nitro-4 hydroxyphenylarsonic acid.

[b]See footnotes a and b, Table 3, page 40.

Management and data collection This experiment was carried out in the same battery room as Experiment II but management and data collection were different. One hundred and twenty 4-week-old male chicks were randomly assigned to two 6-deck batteries so that each pen of chicks had equal weight. The bottom decks were not used in this experiment, because of variation in temperature. Each pen of 6 birds constituted an experimental unit and each dietary treatment was assigned to 4 pens. For DPW re-cycling studies, the experiment was a randomized complete-block arrangement of treatments in a split-plot design with numbers of re-cycling as sub-plots. For urea utilization studies, the experiment was a randomized complete-block design.

Feed and water were available to the chicks ad libitum throughout the four-week test period and lights were turned on at all time. Initial and final weights of feed and birds were recorded for the calculation of weight gain and feed efficiency. For the DPW re-cycling studies, all excreta from each pen was collected and dried weekly for 4 consecutive weeks. Drying process was the same as described in Experiment II. The dried excreta was then ground and mixed with diet to be used for the following period. Part of the dried excreta from each experimental unit was also collected weekly for the determination of its $Cr_2O_3$, minerals and 3-nitro-4-hydroxyphenylarsonic acid contents. Assays for 3-nitro-4-hydroxyphenylarsonic acid were done by the Pharmaceutical Development and Analysis Department of Salsbury Laboratories, Charles City, Iowa. Statistical analysis of the data were completed in the same manner as described for Experiment I.

Results

The summary of data for weight gain and feed efficiency is presented in Table 11 and the corresponding analyses of variance are given in Appendix A, Table 24. Increasing levels of re-cycled DPW in the diets significantly decreased weight gain in linear fashion ($P<0.05$). Re-cycling DPW at the 10% dietary level did not affect feed efficiency but at the 20% dietary level, a 14% depression of feed efficiency was observed. The effect of re-cycling DPW on feed efficiency, however, was not statistically significant. Urea seemed to be utilized to some extent. The response, however, was not statistically significant. Dietary supplemented urea did not affect feed efficiency.

Data for mineral element contents of DPW after re-cycling are shown in Tables 12, 13 and 14. The corresponding analyses of variance are given in Appendix A, Tables 25 and 26. Structural element contents of DPW were significantly affected by the dietary levels of re-cycled DPW. Calcium content of DPW after re-cycling decreased as the levels of re-cycled DPW in the diets increased ($P<0.01$). The reduction of calcium content in DPW was found to be linear and quadratic ($P_{Lin.}<0.01$; $P_{Quad.}<0.05$). Calcium content of DPW also decreased as the numbers of re-cycling increased ($P<0.01$), and the response was also found to be linear and quadratic ($P_{Lin.}<0.01$; $P_{Quad.}<0.05$). Phosphorus content of DPW was affected by the dietary levels of re-cycled DPW ($P<0.01$) but not by the numbers of re-cycle. As the levels of re-cycled DPW increased, phosphorus content of DPW decreased linearly ($P_{Lin.}<0.01$).

Table 11. The effects of urea and re-cycling DPW on weight gain and feed per gain of broilers 4-8 weeks of age - Experiment IV

| Dietary treatments | g. gain/bird[a] | g. feed/g. gain[a] |
|---|---|---|
| Basal diet | 900 | 2.8 |
| Basal + 0.43% urea | 953 | 2.8 |
| Basal + 0.86% urea | 913 | 2.8 |
| 10% DPW | 864 | 2.8 |
| 20% DPW | 782 | 3.2 |

[a]All values represent means of 4 replicate groups, 6 birds per group.

The response of homeostatic elements in DPW to dietary levels of re-cycled DPW seemed to be opposite to that of structural elements. As the dietary levels of re-cycled DPW increased, potassium content of DPW after re-cycling increased significantly in a linear fashion ($P_{Lin.} < 0.01$). Sodium contents of DPW after re-cycling seemed to follow the same response, even though only linear trend was significant ($P_{Lin.} < 0.05$). Number of DPW re-cyclings was not found to affect the homeostatic elements in DPW after re-cycling.

Except iron, all the trace elements in DPW after re-cycling tended to increase linearly ($P_{Lin.} < 0.05$) as the dietary levels of re-cycled DPW were increased. Iron content of DPW decreased significantly in a linear fashion ($P_{Lin.} < 0.01$) as the dietary levels of re-cycled DPW were increased. Only magnesium and zinc contents of re-cycled DPW were found

Table 12. Structural element contents of DPW after re-cycling - Experiment IV

| Elements | Treatments | Number of recycle 1 | 2 | 3 | 4 | Mean |
|---|---|---|---|---|---|---|
| | | Percent[a] in excreta | | | | |
| Ca | 0% DPW | 4.86 | 4.94 | 4.27 | 4.10 | 4.54 |
| | 10% DPW | 3.86 | 3.51 | 2.57 | 3.01 | 3.24 |
| | 20% DPW | 1.41 | 0.70 | 0.56 | 0.44 | 0.78 |
| | Mean | 3.38 | 3.05 | 2.47 | 2.52 | 2.85 |
| P | 0% DPW | 1.87 | 1.96 | 1.87 | 1.89 | 1.90 |
| | 10% DPW | 1.67 | 1.57 | 1.63 | 1.75 | 1.65 |
| | 20% DPW | 1.31 | 1.25 | 1.33 | 1.29 | 1.29 |
| | Mean | 1.62 | 1.59 | 1.61 | 1.64 | 1.62 |

[a]All values represent means of 2 replicate groups, 6 birds per groups.

Table 13. Homeostatic elements of DPW after re-cycling - Experiment IV

| Elements | Treatments | Number of recycle 1 | 2 | 3 | 4 | Mean |
|---|---|---|---|---|---|---|
| | | Percent[a] in excreta | | | | |
| Na | 0% DPW | 0.448 | 0.547 | 0.493 | 0.489 | 0.494 |
| | 10% DPW | 0.610 | 0.662 | 0.638 | 0.475 | 0.596 |
| | 20% DPW | 0.626 | 0.662 | 0.712 | 0.565 | 0.641 |
| | Mean | 0.561 | 0.624 | 0.614 | 0.510 | 0.577 |
| K | 0% DPW | 1.313 | 1.520 | 1.446 | 1.294 | 1.393 |
| | 10% DPW | 1.238 | 1.379 | 1.699 | 1.702 | 1.504 |
| | 20% DPW | 1.884 | 2.472 | 2.191 | 2.758 | 2.326 |
| | Mean | 1.478 | 1.790 | 1.779 | 1.918 | 1.741 |

[a]All values represent means of 2 replicate groups, 6 birds per group.

Table 14. Trace element contents of DPW after re-cycling - Experiment IV

| Elements | Treatments | Number of recycle 1 | 2 | 3 | 4 | Mean |
|---|---|---|---|---|---|---|
| | | Percent[a] in excreta | | | | |
| Mg | 0% DPW | 0.502 | 0.502 | 0.500 | 0.489 | 0.489 |
| | 10% DPW | 0.565 | 0.571 | 0.530 | 0.493 | 0.540 |
| | 20% DPW | 0.580 | 0.606 | 0.627 | 0.541 | 0.588 |
| | Mean | 0.549 | 0.559 | 0.552 | 0.508 | 0.542 |
| Zn | 0% DPW | 0.0306 | 0.0282 | 0.0268 | 0.0274 | 0.0283 |
| | 10% DPW | 0.0458 | 0.0431 | 0.0451 | 0.0376 | 0.0429 |
| | 20% DPW | 0.0621 | 0.0842 | 0.0976 | 0.0918 | 0.0839 |
| | Mean | 0.0462 | 0.0518 | 0.0565 | 0.0523 | 0.0517 |
| Mn | 0% DPW | 0.0410 | 0.0382 | 0.0381 | 0.0418 | 0.0398 |
| | 10% DPW | 0.0466 | 0.0444 | 0.0500 | 0.0419 | 0.0457 |
| | 20% DPW | 0.0499 | 0.0499 | 0.0575 | 0.0534 | 0.0527 |
| | Mean | 0.0458 | 0.0442 | 0.0485 | 0.0457 | 0.0461 |
| Cu | 0% DPW | 0.0031 | 0.0034 | 0.0037 | 0.0033 | 0.0034 |
| | 10% DPW | 0.0044 | 0.0042 | 0.0040 | 0.0036 | 0.0041 |
| | 20% DPW | 0.0049 | 0.0050 | 0.0053 | 0.0054 | 0.0052 |
| | Mean | 0.0041 | 0.0042 | 0.0043 | 0.0041 | 0.0042 |
| Fe | 0% DPW | 0.161 | 0.180 | 0.190 | 0.155 | 0.172 |
| | 10% DPW | 0.143 | 0.134 | 0.138 | 0.138 | 0.138 |
| | 20% DPW | 0.112 | 0.098 | 0.091 | 0.093 | 0.099 |
| | Mean | 0.139 | 0.137 | 0.139 | 0.129 | 0.136 |

[a]All values represent means of 2 replicate groups, 6 birds per group.

to be significantly ($P<0.05$) affected by the number of DPW re-cycling. Magnesium in DPW decreased but zinc in DPW increased linearly and quadratically ($P_{Lin.} \leq 0.05$; $P_{Quad.} \leq 0.05$) after re-cycling. Cubic trend after DPW re-cycling was found to be significant ($P<0.05$) only for manganese content of re-cycled DPW and number of DPW re-cycling x dietary DPW levels interactions were found to be significant ($P<0.01$) for zinc and manganese contents of re-cycled DPW.

Data in Table 15 and analysis of variance in Appendix A, Table 27 show that both dietary levels of DPW and numbers of DPW re-cycling greatly affected percent retention of 3-nitro-4-hydroxyphenylarsonic acid by chicks but there was no statistically significant effect on 3-nitro-4-hydroxyphenylarsonic acid content in dried excreta. However, 3-nitro-4-hydroxyphenylarsonic acid contents in excreta seemed to increase linearly as the levels of re-cycled DPW in the diets increased and quadratically as the number of DPW re-cycling increased. Percentage of intake 3-nitro-4-hydroxyphenylarsonic acid retained by the chicks fed increasing dietary levels of re-cycled DPW decreased significantly ($P<0.01$) in a linear manner ($P_{Lin.} \leq 0.01$). By numbers of DPW re-cycling, the decrease in percent arsonic acid retention by chicks was significant ($P<0.01$) but it was not uniform, since linear, quadratic and cubic trends were found to be significant ($P_{Lin.} \leq 0.01$; $P_{Quad.} \leq 0.01$; $P_{Cub.} \leq 0.01$). The numbers of DPW re-cycling x dietary DPW levels interaction was found to be significant ($P<0.01$) only for 3-nitro-4-hydroxyphenylarsonic acid content in dried excreta.

Table 15. The effects of DPW re-cycling on 3-nitro-4-hydroxyphenylarsonic acid contents in excreta and percent retention - Experiment IV

| Dietary treatments | Number of recycle | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | Mean |
| | PPM in excreta[a] | | | | |
| Basal diet | 41.8 | 44.2 | 41.2 | 38.3 | 41.4 |
| 10% DPW | 38.5 | 53.4 | 50.6 | 42.9 | 46.4 |
| 20% DPW | 35.4 | 53.3 | 54.6 | 49.1 | 48.1 |
| Mean | 38.6 | 50.3 | 48.8 | 43.4 | 45.3 |
| | Percent retained[a] | | | | |
| Basal diet | 75.89 | 73.78 | 75.23 | 74.59 | 74.87 |
| 10% DPW | 77.51 | 70.80 | 74.11 | 73.64 | 74.02 |
| 20% DPW | 72.96 | 68.17 | 68.91 | 67.97 | 69.50 |
| Mean | 75.45 | 70.92 | 72.75 | 72.07 | 72.80 |

[a]All values represent means of 4 replicate groups, 6 birds per group.

## Experiment V

### Objectives

Since the results from Experiment IV showed that chicks fed diets with urea added had better weight gain than those fed the basal diet (16% crude protein), there was a possibility that some of the added urea was utilized by the chicks. This experiment, therefore, was conducted to further explore this possibility. A diet containing 20% DPW was also fed in this experiment to compare with the diet containing 0.86% urea because these diets were equal in total nitrogen.

### General procedure

Diets There were 4 experimental diets (Table 16) in this experiment and each diet was fed to 4 groups of 8 chicks each. DPW used in this experiment was collected from the birds of Experiment III.

Management and data collection This experiment was carried out in the same battery room as was experiment II. One hundred and twenty eight 4-week-old male chicks were randomly distributed to two 6-deck batteries so that each pen had equal weight. The top and the bottom decks were not used in this experiment. The arrangement of treatments in this experiment was a randomized complete-block design with decks of batteries as blocks.

Feed and water were available to the chicks ad libitum throughout the four-week test period and lights were provided at all times. Initial and final weights of feed and birds were recorded, by groups, for the calculations of weight gain and feed efficiency. Statistical

Table 16. Ration composition - Experiment V

| Ingredients | Experimental diets (%) | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| Ground yellow corn | 70.05 | 60.66 | 70.05 | 70.05 |
| Soybean meal (48.5% protein) | 16.20 | 16.34 | 16.20 | 16.20 |
| Alfalfa meal | 8.00 | 2.00 | 8.00 | 8.00 |
| Dicalcium phosphate | 2.45 | - | 2.45 | 2.45 |
| Ground limestone | 2.30 | - | 2.30 | 2.30 |
| Vitamin premix[a] | 0.50 | 0.50 | 0.50 | 0.50 |
| Salt & mineral mix[a] | 0.50 | 0.50 | 0.50 | 0.50 |
| Soybean oil | - | 0.40 | - | - |
| DPW | - | 20.00 | - | - |
| Urea | - | - | 0.86 | 0.43 |
| Calculated analysis: | | | | |
| Approximate true protein (%) | 16.00 | 16.00 | 16.00 | 16.00 |
| Nitrogen (%) | 2.56 | 2.96 | 2.96 | 2.76 |
| M.E. (kcal./kg.) | 2950 | 2953 | 2950 | 2950 |
| Calcium (%) | 1.63 | 1.63 | 1.63 | 1.63 |
| Phosphorus (%) | 0.77 | 0.78 | 0.77 | 0.77 |

[a]See footnotes a and b, Table 3, page 40.

Table 17. The effects of DPW and urea on weight gain and feed per gain of broilers 4-8 weeks of age - Experiment V

| Dietary treatments | g. gain/bird[a] | g. feed/g. gain[a] |
|---|---|---|
| Basal | 939 | 2.66 |
| Basal + 0.43% urea | 934 | 2.70 |
| Basal + 0.86% urea | 931 | 2.80 |
| 20% DPW | 939 | 2.77 |

[a]All values represent means of 4 replicate groups, 8 birds per group.

analyses of the data were completed in the same manner as described in Experiment I.

Results

Data for weight gain and feed efficiency are shown in Table 17 and the corresponding analyses of variance in Appendix A, Table 28. Birds fed the diet containing 20% DPW had the same weight gain as birds fed the control diet but a slight decrease in feed efficiency was obtained with birds fed the 20% DPW diet. Increasing levels of urea in the diets tended to decrease both weight gain and feed efficiency linearly as compared to that obtained from the control group. However, analysis of variance showed no statistical significance for the differences in weight gain and feed efficiency in all cases of dietary treatments.

## Experiment VI

### Objectives

The purpose of this experiment was to investigate the effects of different drug treatments on DPW utilized by broiler-type chicks.

### General procedure

Diets Three ration compositions (Table 18), containing 0, 10 and 20% DPW, were formulated for this experiment. After mixing, each ration was divided into two halves. Amprolium (25%) was added to one-half of the rations at 0.023% and bacitracin (2.2%) to the other half at 0.0034%. Since DPW used in this experiment was collected from laying hens its phosphorus and calcium contents (2.44 and 11.24%) were high which caused the high phosphorus and calcium levels (1.0 and 2.4%) in diets containing 20% DPW. Defluorinated phosphate, potassium phosphate and ground limestone contents of diets containing 0 and 10% DPW were adjusted to keep calcium and phosphorus contents of all experimental diets at the same levels. Diets were isocaloric (2974 kcal./kg.) and were equal in true protein contents (22%).

Management and data collection This experiment was carried out in the same battery room as was Experiment II. One hundred and forty four 4-week-old male chicks were assigned to two 6-deck batteries so that each pen had equal weight. Each pen consisted of 6 birds which represented an experimental unit and each dietary treatment was assigned to four pens. Data collection and other management factors were similar to Experiment V, except that the arrangement of treatments in this

Table 18. Ration composition - Experiment VI

| Ingredients | Experimental diet (%) | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| Ground yellow corn | 51.8 | 45.2 | 38.7 |
| Soybean meal (48.5% protein) | 34.7 | 33.8 | 32.9 |
| Alfalfa meal | 2.0 | 2.0 | 2.0 |
| Defluorinated phosphate | 2.4 | 1.4 | - |
| Ground limestone | 3.9 | 1.8 | - |
| Vitamin premix[a] | 0.5 | 0.5 | 0.5 |
| Salt & mineral mix[a] | 0.5 | 0.5 | 0.5 |
| Soybean oil | 3.2 | 3.8 | 4.3 |
| Potassium phosphate (dibasic) | 1.1 | 1.1 | 1.1 |
| DPW | - | 10.0 | 20.0 |
| Calculated analysis: | | | |
| Approximate true protein (%) | 22.0 | 22.0 | 22.0 |
| M.E. (kcal./kg.) | 2974 | 2973 | 2974 |
| Calcium (%) | 2.4 | 2.4 | 2.4 |
| Phosphorus (%) | 1.0 | 1.0 | 1.0 |

[a]See footnotes a and b, Table 3, page 40.

experiment was a 2 x 3 factorial in a randomized complete-block design.

Results

Data in Table 19 and analysis of variance in Appendix A, Table 29 show that dietary treatments in this experiment significantly ($P<0.01$) affected weight gain and feed efficiency of broiler chicks from 4 to 8 weeks of age. Weight gain of birds fed increasing dietary levels of DPW decreased significantly ($P<0.01$) and linearly ($P_{Lin.} \leq 0.01$). Differences of 48 g. in weight gain between birds fed the control diet and those fed 10% DPW diet and 163 g. between the control group and 20% DPW group were obtained. There was no difference in feed efficiency between birds fed the control diet and the 10% DPW diet but 20% DPW greatly depressed feed efficiency. The decrease in feed efficiency as dietary levels of DPW increased was found to be significantly linear and quadratic ($P_{Lin.} \leq 0.01$; $P_{Quad.} \leq 0.05$).

It was observed that when DPW was supplemented to the diets, birds fed diets supplemented with antibiotic (bacitracin) gained slightly more than birds fed diets supplemented with a coccidiostat (amprolium), even though the difference was not statistically significant. Without DPW added, the diet containing coccidiostat was slightly superior to the diet containing antibiotic for improving weight gain and feed efficiency.

Table 19. The effects of DPW and drug treatments on weight gain[a] and feed per gain[a] of broilers 4-8 weeks of age - Experiment VI

| Drug treatments | DPW (%) | | | Mean |
|---|---|---|---|---|
| | 0 | 10 | 20 | |
| | Gain/bird (g.) | | | |
| Amprolium | 1248 | 1169 | 1047 | 1155 |
| Bacitracin | 1212 | 1195 | 1086 | 1164 |
| Mean | 1230 | 1182 | 1067 | 1160 |
| | g. feed/g. gain | | | |
| Amprolium | 1.78 | 1.83 | 1.98 | 1.86 |
| Bacitracin | 1.82 | 1.79 | 1.94 | 1.85 |
| Mean | 1.80 | 1.81 | 1.96 | 1.86 |

[a]All values represent means of 4 replicate groups, 6 birds per group.

# GENERAL DISCUSSION

## Response of Chicks to Dietary NPN

There are two possible pathways by which chicks could utilize NPN. The first possible pathway is similar to that of ruminants and the general scheme is outlined below:

1. NPN source $\xrightarrow[\text{enzymes}]{\text{microbial}}$ $NH_3$ plus other products
2. Carbohydrates $\xrightarrow[\text{enzymes}]{\text{microbial}}$ Organic acids (including VFA and keto acids)
3. $NH_3$ + keto acids $\xrightarrow[\text{enzymes}]{\text{microbial}}$ Amino acids
4. Amino acids $\xrightarrow[\text{enzymes}]{\text{microbial}}$ Microbial proteins or $NH_3$ plus carbon skeletons
5. Microbial proteins $\xrightarrow[\text{GI. tract}]{\text{enzymes of}}$ Amino acids

Since chickens lack the capacity for microbial fermentation or for microbial synthesis of proteins and amino acids at sites in the alimentary tract which permit their subsequent digestion and efficient absorption, it is postulated that chicks utilize very small amounts of NPN by this pathway.

The second possible pathway is that ammonia from NPN sources enters the portal system and is later changed to glutamate with the aid of ATP. This conversion is known to involve glutamic dehydrogenase which is present in the chick liver. Blair and Young (1970) proved that this pathway could happen in the chick liver.

Response of chicks to dietary NPN was not consistent in these studies. The results obtained from Experiment II indicated that dietary DAC at both 2.87% and 5.70% levels depressed growth and feed efficiency of chicks, but the results of Experiment III showed some beneficial response in weight gain and feed efficiency of birds fed the 2.87% DAC diet. However, one common similarity between the two experiments was that the high level of dietary DAC (5.70%) greatly depressed chick growth and feed efficiency. The deleterious effects of high dietary DAC may be explained by the toxicity of high ammonia levels. In ruminants, microbial synthesis of amino acids from ammonia requires adequate amounts of keto acids from carbohydrates; this concept may apply to chickens. In these experiments, high dietary DAC may have contributed a great amount of ammonia to the chicks so that keto acids contributed from carbohydrates were not enough for microbial synthesis of amino acids, or the rate of ammonia production is much faster than the rate of production of keto acids from carbohydrates. Any of these possibilities may cause a high concentration of ammonia in the intestinal tract of chicks. This ammonia must be excreted in feces, or in uric acid form after synthesis by the liver. The absorption of a large amount of ammonia exposes intestinal, blood vessel and liver tissues to a high ammonia load. Such ammonia load may cause tissue damage or shorten the live span of cells. Evidence that ammonia destroys cells has been reported by Dang and Visek (1968) and Zimber and Visek (1972). Mugerwa and Conrad (1971) also found that high dietary ammonia caused destruc-

tion of intestinal cells which, in turn, caused a rise in metabolic fecal nitrogen. Another explanation for the decrease in growth rate and feed efficiency of chicks fed high dietary DAC is that a high level of ammonia in the diet may cause high concentration of tissue fluid ammonia which may cause metabolic alterations in the chicks. Detoxication of high concentrations of ammonia by the liver may also alter production of metabolites needed by other tissues. Poor palatability of the diet containing DAC might also be a reason for growth depression. Since the result of Experiment III indicated that there was no difference in feed efficiency of birds fed the DAC diet and the control diet during 3-5 weeks of age, the difference in weight gain might be due to lowering of feed consumption.

Nitrogen utilization and nitrogen gain data (Table 6) indicated that the nitrogen from DAC was poorly utilized by chicks. Nitrogen utilized by the chicks fed a 19.32% protein diet was 11.5% greater than that of chicks fed an iso-nitrogenous DAC diet (5.70% DAC). This difference indicates a higher portion of utilizable nitrogen contained in the high-protein basal diet. Similar explanation may be applied to the higher percent nitrogen utilization of chicks fed the low-protein basal diet than that obtained from chicks fed the high-protein basal diet. The increase in percent nitrogen utilization when amino acids were supplemented to the DAC diets might also be explained by the same reasons: highly utilizable amino acids increased the portion of utilizable nitrogen in amino acid-supplemented DAC diets. In fact, the value of

nitrogen utilization and nitrogen gain of chicks fed DAC diets shown in Table 6 may be a little lower than it should be, because damaged tissue from the gastrointestinal tract resulting from a high concentration of ammonia might contribute nitrogen to the fecal nitrogen (see formula for nitrogen utilization, page 44). The differences in percent nitrogen gain might also be explained in this manner.

Birds fed DAC diets seemed to improve their nitrogen utilization and nitrogen gain with age. Graphical illustrations in Figures 3 and 4 show that birds fed DAC diets improved nitrogen utilization and nitrogen gain up to four weeks of age, but at five weeks a drastic drop occurred. This decrease of nitrogen gain and nitrogen utilization may be due to two possibilities. First, there was a possibility of tissue damage due to ammonia overload. Secondly, protein requirement for tissue formation of birds after four weeks of age decreased. The decrease after four weeks of age may also be due to the combination of both possibilities.

It was interesting that birds fed the 19.32% crude protein basal diet gained less than birds fed the 15.11% crude protein basal diet, but they had better feed efficiency. The reason for this difference might be explained by the different energy sources contained in the two diets. Dextrose (21.78%) and a high level of soybean oil (5.78%) might have increased the palatability of the 15.11% crude protein basal diet which, in turn, caused the birds to consume more and gain more even though their feed efficiency was lower.

The results from both Experiment II and Experiment III indicated that amino acid supplementation of DAC diets improved growth rate and

feed efficiency of the birds. The supplemented amino acids might contribute more utilizable nitrogen to the DAC diet and also might improve diet efficiency by balancing the amino acids in the diet, because both weight gain and feed efficiency were greatly improved by the amino acids supplementation. However, supplemented amino acids did not entirely remove the growth depressing effect of DAC, because birds fed the 15.11% protein basal diet supplemented with amino acids gained more than did birds fed the amino acid-supplemented DAC diet, as shown in Experiment III (Table 9).

Dietary urea seemed to depress growth and feed efficiency in Experiment V. On the contrary, growth rate of birds fed urea diets in Experiment IV were significantly improved, but feed efficiency was not. The difference might be due to the 3-nitro-4-hydroxyphenylarsonic acid in the diets of Experiment IV. It has been mentioned that a high level of ammonia in the diet may cause toxicity in chicks. Microbial enzyme activity might be decreased by 3-nitro-4-hydroxyphenylarsonic acid which may decrease the rate of ammonia production from urea. The decrease in rate of ammonia released from urea might decrease ammonia toxicity and also increased ammonia utilization by microbial flora for amino acid synthesis in the gastrointestinal tract of chicks. This might be one of the reason why birds fed the diet with urea gained more than those fed the basal diet in Experiment IV. Evidence to support this explanation could not be found in the literature for chicks, but many investigations in this area have been done with mammals. Prescott (1953)

observed that antibiotics depressed urease activity in rumen fluid and Visek et al. (1959) found that a growth promoting concentration of antibacterial agents inhibited microbial urease synthesis in the alimentary tract of rats. Brown et al. (1960) found that calves made satisfactory gain on rations containing 3% less protein equivalent if antibiotics were included in their diet. Derivatives of hydroxamic acid decreased ruminal ammonia production and improved nitrogen retention in ruminants (Streeter et al., 1969).

## Response of Chickens to Dietary DPW

Five experiments were conducted to investigate the response of chickens to dietary DPW. The performance of laying hens fed DPW diets was observed in Experiment I. Egg production on a hen-day basis and percentage of hens out of production are given graphically in Figures 1 and 2, respectively. DPW decreased egg production and feed consumption of hens. Consequently, egg weight increased as the dietary level of DPW increased because egg weight normally increases when egg production rate decreases. This finding is confirmed by the result of Flegal and Zindel (1969, 1970b) who reported that egg production on a hen- housed basis decreased as the levels of DPW in the diets increased from 10 to 30%, and the 30% level of DPW significantly increased the feed per dozen eggs. However, Flegal and Zindel (1970a), Hodgetts (1971), York et al. (1970), Nesheim (1972), Young (1972) and Young and Nesheim (1972) did not find any deleterious effect of DPW on laying hens. In contrast, Blair and Lee (1973) found improvement of laying hen per-

formance when the DPW diets were supplemented with essential amino acids.

Percentage of hens out of production seemed to increase as the dietary level of DPW increased. This may be related to the decrease in daily feed consumption which, in turn, correlated with the palatability and texture of the feed. Dustiness, high NPN and high fiber contents of DPW might reduce the palatability of DPW diet. Approximately 56% of the total nitrogen content of DPW is NPN, this NPN contributed a considerable amount of ammonia and a possibility of ammonia toxicity should also be taken into account. High percent mortality of birds fed DPW diets might be an evidence of ammonia toxicity or this may have been caused by low energy intake.

Egg production and percentage of hens out of production decreased while egg weight increased by periods, these effects being rather consistent regardless of dietary treatments. In fact, birds normally give similar response as they get older.

Responses of young chicks to dietary DPW were investigated in Experiments II, IV, V and VI. Without re-cycling, DPW at a dietary level of 10% did not affect weight gain and feed efficiency of young chicks, but 20% of DPW in the diets greatly depressed growth and feed efficiency. These results were similar to those reported by Flegal and Zindel (1970c). Since the diets used in these experiments were formulated on a true protein basis, it might be concluded that on the overall average basis, young chicks could utilize NPN to a very small extent (or not at all) when DPW was supplemented to the diets at a low level (10%). But at a

high dietary level of DPW (20%), ammonia toxicity might occur in the chicks. Nitrogen utilization and nitrogen gain shown graphically in Figures 3 and 4 indicate that chicks could improve their utilization and gain of nitrogen in DPW diets by age: nitrogen utilization and nitrogen gain of birds fed DPW diets increased from 2 to 4 weeks of age, but after 4 weeks of age, both nitrogen utilization and nitrogen gain dropped drastically. This drop must be a sign of tissue damage caused by ammonia toxicity or a sign of decrease in protein requirement with age or due to combinations of both possibilities.

With re-cycling, DPW at a dietary level of 10 or 20% greatly depressed growth. There was no effect of 10% DPW on feed efficiency but 20% DPW greatly depressed feed efficiency (Experiment IV). The reason for the growth-depressing effect of both low and high dietary levels of re-cycled DPW could be explained by the decrease in calcium content and slight increase in phosphorus content of DPW after re-cycling (Table 12). According to diet formulation (Table 10), DPW was used as the sole source of calcium and phosphorus in the 20% DPW diet and partly in 10% DPW diet. Any change in the calcium and phosphorus ratio in DPW would also alter the ratio of calcium and phosphorus in the diet, which, in turn, could depress growth and feed efficiency of the birds. The reason for reduction in calcium content of DPW after re-cycling could be due to high requirement of calcium for structure formation of young chicks. It is well known that a high calcium level in the diet competes with phosphorus for absorption by the chicks and it also prevents phosphorus from

being absorbed by precipitating the phosphorus. This might explain why phosphorus content of DPW after re-cycling slightly increased, since calcium to phosphorus ratio in the diets of this experiment was greater than normal. On the contrary, Flegal and Dorn (1971) reported that continuously re-cycling DPW in laying diets resulted in a slight accumulation of calcium and phosphorus.

Other mineral contents of DPW, except iron, seemed to be constant after re-cycling. This may be due to a high requirement of iron for hemoglobin formation. As the level of re-cycled DPW in the diet increased, a trend of increase in other mineral contents of DPW was observed. Constant requirement of these minerals by the chicks might be the explanation for the increase when dietary levels of re-cycled DPW were increased.

With re-cycling, the amount of 3-nitro-4-hydroxyphenylarsonic acid in the excreta seemed to increase quadratically but percentage of 3-nitro-4-hydroxyphenylarsonic acid intake retained by the birds decreased. As the dietary level of re-cycled DPW increased, the amount of 3-nitro-4-hydroxyphenylarsonic acid in the excreta also increased but percentage retained by the birds decreased (Table 15). The results indicated that birds tended to retain a constant amount of 3-nitro-4-hydroxyphenylarsonic acid, but during the first two weeks of DPW re-cycling, the birds tried to excrete more and, thus, retained less of the arsonic acid. There is a possibility of 3-nitro-4-hydroxyphenylarsonic acid accumulation in the birds' bodies but not in the excreta.

Other investigators (Mameesh et al., 1959; Warden and Schaible, 1961)

have reported that addition of antibiotics to diets contaminated with hen feces improved growth and feed efficiency of young chicks. No previous work was found that investigated the different effects of antibiotics and coccidiostats on chicks fed diets supplemented with DPW. Experiment VI was designed to serve this purpose. The result in Table 19 indicates that an antibiotic (bacitracin) was slightly superior to a coccidiostat (amprolium) in promoting weight gain and feed efficiency when diets contained DPW. Without DPW, the diet containing coccidiostat seemed to be superior. However, the difference was not statistically significant. It is possible that the antibiotic promoted the utilization of NPN from the DPW diet.

# CONCLUSIONS

1. Diammonium citrate (DAC) and urea were not utilized by young chicks when supplemented at dietary level of 2.85% or 5.70% for DAC and 0.43% or 0.86% for urea to a 15% protein practical diet containing no antibiotic.
2. With a diet containing an antibiotic, a portion of the nitrogen from DAC and urea are utilized by the chicks when these NPN sources are supplemented to a 15% protein practical diet at levels of 2.85% for DAC and 0.43% for urea.
3. At the dietary level of 5.7% for DAC and 0.86% for urea, chick growth and feed efficiency were depressed, probably because of ammonia toxicity.
4. DAC in the diets does decrease nitrogen utilization and nitrogen gain of the chicks.
5. Supplementation of amino acids to diets containing DAC improves weight gain, feed efficiency, nitrogen utilization and nitrogen gain of the chicks.
6. Chicks fed DAC diets improve percent nitrogen utilization and nitrogen gain by age.
7. Dried poultry waste (DPW) in the diets at 12.5 or 25.0% decreases egg production, and increases percentage of hens out of production and percent mortality.

8. Supplementation of 10% DPW to a 15.0% protein diet does not affect weight gain and feed efficiency of young chicks, but 20% dietary DPW depresses growth and feed efficiency.
9. With re-cycling, DPW at both 10% and 20% dietary levels, greatly depresses chick growth, but feed efficiency is depressed by the diet containing 20% DPW only.
10. After DPW re-cycling, calcium and iron contents of the re-eycled DPW decrease. Other minerals (Na, K, Mg, Zn, Mn and Cu) contents are constant.
11. DPW re-cycling may cause accumulation of 3-nitro-4-hydroxyphenyl-arsonic acid in the chicks' bodies, but not in the excreta.
12. Antibiotic (bacitracin) is slightly superior to coccidiostat (amprolium) in promoting weight gain and feed efficiency of birds fed diets containing DPW.
13. DPW could be used as feedstuff for chickens at a dietary level of 10%, but antibiotics should be supplemented.

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# APPENDIX A

Table 20. Analysis of variance of feed per hen per day, percent egg production, percent hens out of production and average egg weight - Experiment I

| Source of variation | d.f. | M.S. Feed per hen per day | M.S. % egg production | M.S. % hens out of production | d.f. | M.S. Average egg weight |
|---|---|---|---|---|---|---|
| Replicate | 3 | 54 | 15 | 91 | 3 | 7 |
| Treatment | 2 | 117 | 170* | 46 | 2 | 10 |
| Linear | (1) | 232** | 328** | 92 | (1) | 14** |
| Quadratic | (1) | 1 | 12 | 0 | (1) | 6 |
| Error (a) | 6 | 94 | 22 | 79 | 6 | 4 |
| Period | 4 | 134** | 358** | 1064** | 3 | 81** |
| Linear | (1) | 21 | 1264** | 3639** | (1) | 207** |
| Quadratic | (1) | 129* | 17 | 602* | (1) | 18** |
| Cubic | (1) | 340** | 149 | 6 | (1) | 18** |
| Period x treatment | 8 | 57** | 28 | 137 | 6 | 1 |
| Error (b) | 36 | 21 | 16 | 121 | 27 | 1 |

*Probability 0.05 or less here and throughout

**Probability 0.01 or less here and throughout

Table 21. Analysis of variance of weight gain and feed per gain - Experiment II

| Source of variation | d.f. | M.S. Weight gain | M.S. Feed/gain |
|---|---|---|---|
| Replicate | 3 | 2005 | 0.0002 |
| Treatment | 9 | 27923** | 0.1272** |
| N source (3,4,5,6 vs. 7,8,9,10) | 1 | 58300** | 0.0005 |
| DPW level (2 vs. 3 vs. 5) | 2 | 15023** | 0.0403** |
| Linear | (1) | 19562** | 0.0512** |
| Quadratic | (1) | 10488** | 0.0294* |
| DAC level (2 vs. 7 vs. 9) | 2 | 45261** | 0.0502** |
| Linear | (1) | 86445** | 0.0924** |
| Quadratic | (1) | 4077 | 0.0081 |
| Amino acid (3,5,7,9 vs. 4,6,8,10) | 1 | 129872** | 0.5805** |
| Error | 27 | 1273 | 0.0056 |

Table 22. Analysis of variance of percent nitrogen utilization and percent gain - Experiment II

| Source of variation | d.f. | M.S. % N utilization | M.S. % N gain |
|---|---|---|---|
| Replicate | 3 | 13.25 | 3.83 |
| Treatment | 9 | 430.62** | 354.75** |
| N source (3,4,5,6 vs. 7,8,9,10) | 1 | 555.64** | 1191.40** |
| DPW level (2 vs. 3 vs. 5) | 2 | 427.47** | 219.78** |
| Linear | (1) | 753.96** | 351.55** |
| Quadratic | (1) | 99.75 | 87.77* |
| DPW level (2 vs. 7 vs. 9) | 2 | 1401.43** | 1039.58** |
| Linear | (1) | 2313.90** | 1599.29** |
| Quadratic | (1) | 488.67* | 479.04** |
| Amino acid (3,5,7,9 vs. 4,6,8,10) | 1 | 415.58** | 531.19** |
| Error (a) | 27 | 29.91 | 16.38 |
| Period | 3 | 391.87** | 508.17** |
| Linear | (1) | 262.39** | 393.23** |
| Quadratic | (1) | 394.13** | 362.77** |
| Cubic | (1) | 519.19** | 768.08** |
| Period x treatment | 27 | 54.29** | 47.23** |
| Error (b) | 90 | 23.80 | 15.84 |

Table 23. Analysis of variance of weight gain and feed per gain of birds 1-3 weeks and 3-5 weeks of age - Experiment III

| Source of variation | d.f. | Weight gain | | Feed per gain | |
|---|---|---|---|---|---|
| | | 1-3 weeks M.S. | 3-5 weeks M.S. | 1-3 weeks M.S. | 3-5 weeks M.S. |
| Replicate | 3 | 160 | 1116 | 0.0025 | 0.0051 |
| Treatment | 5 | 2346** | 6352** | 0.0329** | 0.0515** |
| Amino acid level | 1 | 2420** | 10174** | 0.0938** | 0.2282** |
| DAC level | 2 | 4133** | 8292** | 0.0284** | 0.0110 |
| Linear | (1) | 6006** | 9859** | 0.0306** | 0.0002 |
| Quadratic | (1) | 2260** | 6725** | 0.0261** | 0.0217* |
| Amino acid x DAC level | 2 | 522* | 2500* | 0.0070 | 0.0038 |
| Error | 15 | 93 | 572 | 0.0019 | 0.0033 |

Table 24. Analysis of variance of weight gain and feed per gain - Experiment IV

| Source of variation | d.f. | M.S. | |
|---|---|---|---|
| | | Weight gain | Feed per gain |
| Replicate | 3 | 2430 | 0.0261 |
| Treatment | 4 | 16620* | 0.6207* |
| DPW level (1 vs. 2 vs. 3) | 2 | 14556* | 0.2250 |
| Linear | (1) | 27691* | 0.3741 |
| Quadratic | (1) | 1420 | 0.0715 |
| Urea level (1 vs. 4 vs. 5) | 2 | 3102 | 0.0016 |
| Linear | (1) | 334 | 0.0032 |
| Quadratic | (1) | 5869 | 0.0000 |
| Error | 12 | 3464 | 0.1700 |

Table 25. Analysis of variance of structural element content and homeostatic element content of DPW after re-clying - Experiment IV

| Source of variation | d.f. | Structural elements | | Homeostatic elements | |
|---|---|---|---|---|---|
| | | Ca M.S. | P M.S. | Na M.S. | K M.S. |
| Replicate | 1 | 0.238 | 0.0062 | 0.0021 | 0.0115 |
| DPW level | 2 | 29.378** | 0.7389** | 0.0453 | 2.0769* |
| Linear | (1) | 56.490** | 1.4593** | 0.0863* | 3.4806** |
| Quadratic | (1) | 2.266* | 0.0185 | 0.0044 | 0.6735 |
| Error (a) | 2 | 0.066 | 0.0044 | 0.0040 | 0.0745 |
| Number of recycle | 3 | 1.018** | 0.0025 | 0.0166 | 0.2082 |
| Linear | (1) | 2.355** | 0.0026 | 0.0080 | 0.5125 |
| Quadratic | (1) | 0.432* | 0.0048 | 0.0417* | 0.0446 |
| Cubic | (1) | 0.267 | 0.0001 | 0.0002 | 0.0675 |
| Number of recycle x DPW level | 6 | 0.166 | 0.0075 | 0.0041 | 0.1026 |
| Error (b) | 9 | 0.081 | 0.0052 | 0.0048 | 0.1278 |

Table 26. Analysis of variance of trace element contents of DPW after re-cycling - Experiment IV

| Source of variation | d.f. | M.S. x $10^4$ | | | | |
|---|---|---|---|---|---|---|
| | | Mg | Zn | Mn | Cu | Fe |
| Replicate | 1 | 1.28 | 0.002 | 0.0973 | 0.00487 | 0.175 |
| DPW level | 2 | 163.56 | 66.666 | 3.4305* | 0.06040 | 107.733* |
| Linear | (1) | 326.40* | 123.960* | 6.6800* | 0.12000* | 215.040** |
| Quadratic | (1) | 0.60 | 9.360 | 0.1600 | 0.00250 | 0.440 |
| Error (a) | 2 | 9.13 | 5.399 | 0.0933 | 0.00344 | 2.068 |
| Number of recycle | 3 | 32.86* | 1.077* | 0.0755 | 0.00084 | 1.472 |
| Linear | (1) | 51.72* | 1.580* | 0.0150 | 0.00002 | 2.388 |
| Quadratic | (1) | 45.60* | 1.470* | 0.0030 | 0.00163 | 1.199 |
| Cubic | (1) | 1.30 | 0.190 | 0.2070* | 0.00085 | 0.828 |
| Number of recycle x DPW level | 6 | 10.64 | 2.040** | 0.1660** | 0.00193 | 2.996 |
| Error (b) | 9 | 5.90 | 0.169 | 0.0212 | 0.00064 | 2.070 |

Table 27. Analysis of variance of PPM in dried excreta and percent retention of 3-nitro-4-hydroxyphenylarsonic acid after re-cycling - Experiment IV

| | | M.S. | |
|---|---|---|---|
| Source of variation | d.f. | PPM in excreta | % retention |
| Replicate | 3 | 270.5 | 6.8 |
| DPW level | 2 | 193.9 | 133.0** |
| Linear | (1) | 359.1 | 230.5** |
| Quadratic | (1) | 28.6 | 35.5 |
| Error (a) | 6 | 118.2 | 6.1 |
| Number of recycle | 3 | 8.8 | 44.6** |
| Linear | (1) | 10.7 | 41.7** |
| Quadratic | (1) | 4.5 | 44.6** |
| Cubic | (1) | 11.1 | 47.4** |
| Number of recycle x DPW level | 6 | 30.6** | 5.5 |
| Error (b) | 27 | 7.0 | 2.6 |

Table 28. Analysis of variance of weight gain and feed per gain - Experiment V

| Source of variation | d.f. | M.S. | |
|---|---|---|---|
| | | Weight gain | Feed per gain |
| Replicate | 3 | 870.00 | 0.0046 |
| Treatment | 3 | 61.00 | 0.0164 |
| DPW level (1 vs. 2) | 1 | 0.72 | 0.0221 |
| Urea level (1 vs. 4 vs. 3) | 2 | 59.78 | 0.0217 |
| Linear | (1) | 120.75 | 0.0406 |
| Quadratic | (1) | 0.63 | 0.0026 |
| Error | 9 | 1150.00 | 0.0129 |

Table 29. Analysis of variance of weight gain and feed per gain - Experiment VI

| Source variation | d.f. | M.S. | |
|---|---|---|---|
| | | Weight gain | Feed per gain |
| Replicate | 3 | 0.00177 | 0.00383 |
| Treatment | 5 | 0.02400** | 0.02760** |
| DPW level | 2 | 0.05615** | 0.06430** |
| Linear | (1) | 0.10628** | 0.11611** |
| Quadratic | (1) | 0.00603 | 0.02935* |
| Drug | 1 | 0.00051 | 0.00204 |
| Drug x DPW level | 2 | 0.00323 | 0.00405 |
| Error | 15 | 0.00258 | 0.00572 |

# APPENDIX B

## Analytical Procedures

### Determination of chromic oxide

Chromic oxide was determined by the method described by Bolin *et al.* (1952).

Preparation of oxidizing reagent Ten grams of sodium molybdate was dissolved in 150 ml. of distilled water, and 150 ml. of concentrated sulfuric acid was added slowly while the solution was cooled in an ice bath. Slowly, and with frequent stirring, 200 ml. of 70-72% perchloric acid was added to the mixture.

Procedure Three hundred mg. of dried sample which had been ground through a 40-mesh sieve as was transferred to a dry 100-ml. Kjeldahl flask. Three glass beads and 5 ml. of oxidizing reagent were added to wash any adhering particles down the side of the flask. The flask was heated over a micro burner until a clear digestion mixture was obtained. In the oxidation of some samples, black particles adhered to the neck and sides of the flask. In this case, the flask was turned 180° and the sample was allowed to be digested 2 or 3 minutes longer. After a clear mixture was obtained, the burner was turned off and 2 ml. of 70-72% perchloric acid was added to the digestion mixture. The mixture was reheated for 10 minutes, then was allowed to cool slowly to room temperature. Fifty ml. of distilled water was added to the mixture and the mixture was diluted to 100 ml. with distilled water in a 100-ml. volumetric flask and allowed to stand for 10 minutes to allow silica to settle. The concentration of chromic oxide was read against

distilled water at 440 mu in a micro-sample spectrophotometer (Gilford 300-N) which was pre-adjusted with known concentration standard solutions of chromic oxide (concentration ranged from 10 to 120 mg. per ml.).

Determination of minerals in dried excreta

The analytical procedure was that described by Ewan[1].

Reagents 1) Concentrated nitric acid; 2) 70-72% perchloric acid; 3) 5N. hydrochloric acid - approximately 431 ml. of concentrated hydrochloric acid was diluted to 1000 ml. with deionized water; 4) 10% lanthanum chloride - 100 g. of lanthanum chloride was dissolved in deionized water containing 8.6 ml. of concentrated hydrochloric acid and the solution was diluted to 1000 ml. with deionized water.

Procedure One gram of sample, 3 glass beads and 15 ml. of concentrated nitric acid were transferred to a dry 100-ml. Kjeldahl flask. It was allowed to stand overnight, then the sample was slowly heated to boil until about one-half of the nitric acid was distilled off. The flask was then removed from the heat and allowed to cool for 5-10 minutes before 8 ml. of concentrated perchloric acid was added. Heating was resumed for 30 minutes or until the last of nitric acid left the digest. This might be accompanied by rapid oxidation (boiling), clearing of the digest and the appearance of white fume. The flask was removed from the heat and was allowed to cool for 10 minutes before

[1]R. C. Ewan, 337 Kildee, Iowa State University, Ames, Iowa. Personal communication, 1974.

3 ml. of 5N hydrochloric acid was added. Heating was resumed. After the hydrochloric acid and water was driven off, which accompanied by white fumes condensing in the neck of the flask, the heating was continued for 10 minutes. Then the digest was removed from the heat, cooled and transferred quantitatively to a volumetric flask. The dilution factors for different minerals are shown below:

| Mineral | Dilution factor |
|---|---|
| Cu | 25 |
| Fe, Mn | 250 |
| Zn | 1250 |
| Mg*, Ca*, Na, K | 12500 |

*One ml. of 10% lanthanum chloride was added to every 10 ml. of dilution.

Optical density readings were obtained by atomic absorption spectrophotometer (Techtron model AA-5) at various settings as follow:

| Mineral | Lamp current (ma.) | Wavelength (Å) | Slit (micron) |
|---|---|---|---|
| Cu | 3 | 3247.5 | 100 |
| Fe | 10 | 2483.3 | 100 |
| Mn | 10 | 2794.8 | 50 |
| Zn | 5 | 2138.6 | 100 |
| Mg | 5 | 2852.1 | 100 |
| Ca | 5 | 4226.7 | 100 |
| Na | 10 | 5870.0 | 100 |
| K | 10 | 7664.9 | 300 |

The readings were compared with standard curves which were prepared following the above procedure by plotting optical density against different mineral concentrations.

Determination of phosphorus in dried excreta

Phosphorus content of excreta was determined by the method described by Ewan[1].

Reagents 1) Digestion reagent: 10 g. of sodium molybdate was dissolved in 150 ml. of deionized water. One hundred and fifty ml. of concentrated sulfuric acid was added to the solution slowly and allowed to cool, then 200 ml. of 70-72% perchloric acid was added. 2) Molybdovanadate reagent: 20 g. of ammonium molybdate was dissolved in 200 ml. of hot deionized water and allowed to cool. One g. of ammonium metavanadate was dissolved in 125 g. of hot deionized water and 225 ml. of 70-72% perchloric acid added to the solution. Molybdate solution was then added to the vanadate solution gradually with stirring. The obtained mixture was then diluted to 2 litres.

Procedure Half a gram of sample, 10 ml. of digestion reagent and 3 glass beads were transferred to a dry 100-ml. Kjeldahl flask. The sample was heated until it turned light green and the color persisted for approximately 10 minutes. The digest was allowed to cool before it was quantitatively transferred to a 500-ml. volumetric flask and diluted to the mark with deionized water. Five ml. aliquots of the dilution were then transferred in duplicate to 18 x 150 mm. test tubes. Two ml. of molybdovanadate reagent and 3 ml. of deionized water were added to the aliquots. The test tubes were allowed to stand for

[1]R. C. Ewan, 337 Kildee, Iowa State University, Ames, Iowa. Personal communication.

10 minutes before concentration readings were taken by using micro-sample spectrophotometer (Gilford 300-N) which was pre-adjusted with known concentration standard solution of phosphorus (concentrations ranged from 1 to 6 mcg. per ml.). The reading was made at 400 mu.